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SURVIVAL OF PATHOGENS IN ANIMAL MANURE DISPOSAL

Stanley L. Diesch, et al

Minnesota University

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Model oxidation ditches were used in laboratory studies. At simulated summer and winter environmental conditions determinations were made of the viability and infectivity of leptospires in weanling hamsters and salmonella in turkey poults. Salmonella was transmitted by aerosols, but leptospires were not. In refeeding contaminated slurry contents salmonella was transmitted but leptospires not. Leptospires isolated from the slurry of the model ditch 17 days post seeding had lost measurable virulence.

Measurements of selected microbial aerosols were made in the vicinity of a field ditch. Bacterial levels of 100-200 total colony-forming units per liter of air were associated with the beef cattle population in the housing unit and not with aerosols generated by the oxidation ditch treatment system.

Studies were made on a model oxidation ditch simulating the field ditch. The winter temperature conditons (2° - 5° C) slowed the degradation process considerably and high dissolved oxygen was maintained.

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SURVIVAL OF PATHOGENS
IN
ANIMAL MANURE DISPOSAL

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A research project was conducted to measure and evaluate the public health effects of pathogens in beef cattle manure found in the extended aeration system of waste disposal.

Model oxidation ditches were used in laboratory studies. At simulated summer and winter environmental conditions, determinations were made of the viability and infectivity of leptospires in weanling hamsters and salmonella in turkey poults. Salmonella was transmitted by aerosols, but leptospires were not. In refeeding contaminated feed and slurry contents, salmonella was transmitted but leptospires were not. Leptospires isolated from the slurry of the model ditch 17 days post seeding had lost measurable virulence.

Measurements of selected microbial aerosols were made in the vicinity of a field ditch. Bacterial levels of 100-200 total colony-forming units per liter of air were associated with the beef cattle population in the housing unit and not with aerosols generated by the oxidation ditch treatment system.

Studies were made on a model oxidation ditch simulating the field ditch. The winter temperature conditions (2°-5° C) slowed the degradation process considerably and high dissolved oxygen was maintained.

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SECTION I

CONCLUSIONS

Further refinement of simulation of the oxidation ditch field conditions in the laboratory was accomplished in this two-year project concerned with survival of pathogens in animal manures. A mechanism was developed to simulate transmission of pathogenic microorganisms from the laboratory model oxidation ditch to laboratory animals housed above the ditch over the wheel-like rotor.

Improved methods of detection of leptospires in use and further refinement of the agar plate method were developed.

Quantitation studies indicated salmonella died (decimal reduction) and leptospires lived or multiplied in the manure slurry environment of the laboratory oxidation ditch.

In aerosol studies in the laboratory, leptospires were detected in the air on one occasion and salmonella several times. Leptospires were not transmitted to hamsters by recycled feed that was gathered from leptospiral-contaminated manure slurry nor were the hamsters infected via aerosols. Salmonella was transmitted to turkey poults by feeding salmonella-contaminated feed and via aerosols.

There is some evidence that virulent leptospires exposed to an aerated manure slurry environment in an oxidation ditch lose their virulence and ability to infect or cause an antibody response in weanling hamsters. Further work is needed.

Standard procedures for aerosol sampling at warm and cold environmental temperatures were developed.

The air environment within a field beef-housing unit harbored a rich bioaerosol of microorganisms. Counts of approximately 100 to 200 total colony-forming units per liter of air sampled were observed in the year of air sampling. It was concluded that these high levels were associated with the beef cattle population housed within the oxidation ditch unit and not with the oxidation ditch treatment system.

It was concluded that wet-floor environment in the buildings suppressed aerosol formation. During cleaning periods with a dry floor, unusually high, potentially hazardous, aerosol levels existed within the barns.

Pathogens (leptospires and salmonella) were not isolated in the aerosol sampling of the field project. However, there was no evidence that the animals were infected.

Total plate counts downwind for aerobic organisms were higher (6 versus 1.7 CFU/l) when the animals were present probably due to increased wind turbulence. This conclusion was based on the information that the coliform counts were still zero downwind when the cattle were present. Also, the fecal streptococci were only sporadically found and were, on the average, at the lower level of 0.04 CFU/l. None were detected at the 160-cm level. Most of the total counts were probably aerobic soil microorganisms which had their source in fields upwind from the sampling site and not from the oxidation ditch.

In Model B laboratory model studies, the 20° C ditch temperature promoted faster breakdown of the animal waste than the 10° C temperature which, in turn, was more than the 2° C breakdown rate. Rotor speed had an effect on breakdown only at the 20° C temperature, when there was a high demand for oxygen by the microorganisms. In a field oxidation ditch, the information gained in this research could be used to significantly change the management of this system. During cold weather, the rotor speed could be reduced with a significant savings in energy and maintenance costs. The dissolved oxygen levels can be maintained at a level high enough to promote aerobic degradation and minimize odor.

SECTION II

RECOMMENDATIONS

There is evidence that salmonella and leptospires survive for long periods of time in the manure slurry environment (this was based on short-term, 5-day, seeding of virulent pathogens) and it has been documented that infected cattle may shed leptospires in urine and salmonella in feces for weeks to months, the slurry becomes an environment to maintain viable pathogens. Therefore, we recommend that manure effluent and sludge containing pathogens (leptospires and salmonella) should be treated to kill the microorganisms.

There was evidence that leptospires lose their virulence and are unable to infect when exposed to a manure environment. We recommend further studies.

A high level of bacteria was found in aerosols of the field housing unit during cleaning activity; face masks and protective clothing are recommended.

Because the low temperature studies showed slow rates of degradation, some field trials with slow rotor speeds should be attempted to see if low odor levels are maintained. The limit of this recommendation was tried during the winter of 1973-1974 at the Rosemount field ditch. The rotor was shut off for the winter. Odors were not offensive and considerable savings in energy and maintenance were achieved.

SECTION III

INTRODUCTION

Today, quality of environment and ecology are of vital concern to every segment of society. In the U.S. the total volume of animal waste is estimated at 1.5 million tons of which more than half is produced by concentrated systems. Animal wastes are of concern in the abatement of water, air, and soil pollution and the dissemination of infectious agents from animals to animals and man.

In nature, organisms which are pathogenic to man and animals may be present in the excreta of domestic and wild animals. More than 150 diseases of animals are transmitted to man. Some of the most significant bacterial zoonoses transmitted by animal wastes are salmonelloses, staphyloccal and streptococcal infections, tetanus, brucellosis, tuberculosis, leptospirosis and colibacillosis.

A literature survey concerned with the recovery of specific microorganisms from urine and feces of inoculated infected animals indicated that 14 microorganisms of specific disease entities were recovered from the feces of infected cattle (anthrax, brucellosis, foot and mouth, leptospirosis, psittacosis-ornithosis, Q fever, rinderpest, tuberculosis, tularemia, and adenovirus, Coxsachie A and Coxsachie B virus, enterovirus, and reovirus infections) and 7 microorganisms of disease from the urine of inoculated infected cattle (brucellosis, foot and mouth, leptospirosis, Q fever, rinderpest, tuberculosis and tularemia). The survey excluded most intestinal diseases (1).

In an extensive literature survey of solid waste/disease relationships, Hanks (2) states that the literature fails to supply data which would permit a quantitative estimate of relationship between solid waste and disease. However, he further states that circumstantial and epidemiologic information presented in reports does support the definite relationship of disease and solid wastes. He further states that in developed countries, incidence, prevalence, and severity of human infection due to animal fecal wastes are low from the standpoint of reported outbreaks, but suspicion is that the amount of disease is actually much higher.

This research project was a continuation of a study made over a three-year period concerned with the survival of pathogens in animal manure disposal. The major purposes of the initial three-year study were: to measure survival of Leptospira pomona and Salmonella typhimurium in beef cattle manure under specific, measured environmental and physical conditions; to compare methods for measuring, detecting and preventing survival of pathogenic bacteria (leptospires and salmonella) in animal manure; to simulate, produce and maintain field environmental conditions in the laboratory; and to establish criteria in the hydraulic and structural design of oxidation channels, vertical aerators, other forms of extended aeration devices, permissible loading rates of solid waste into aeration devices, especially at warm and cold temperatures, and the effect on survival of pathogens (3,4,5,6) (refer to Final Report, Survival of Pathogens in Animal Manure Disposal, EP-00302, 1971).

This two-year research was designed to measure and evaluate the public health effect of specific pathogens in beef cattle manure found in the extended aeration system of waste disposal and if potential pollution of the common environment of man and animals occurs.

The purpose of this study was to determine and evaluate (a) the public health hazards associated with potential pathogen transmission from the internal and external enviroment and from feed recycled from animal manure disposal during aerobic treatment. Determinations were made of the viability and infectivity of leptospires and salmonella in aerosols caused by potential mechanical dissemination of these pathogens from manure of a model oxidation ditch. Viability was measured in cultural media and infectivity in laboratory animals; (b) selected microbial aerosols generated during aerobic treatment of animal manures in an oxidation ditch under a beef confinement housing unit. Environmental samplings of aerosols and culturing of fecal-borne bacteria were made in proximity to the field ditch; (c) relationships between temperature, loading rates and degradation of manure in a model oxidation ditch were made under controlled environment simulating the field ditch and further utilized to develop design of the oxidation ditch. This research was conducted in the laboratory and the field by the research team from Veterinary Medicine, Agricultural Engineering, and Public Health, University of Minnesota.

More specific information will be found at the beginning of each of the following sections.

SECTION IV

LABORATORY MODEL PATHOGEN STUDIES

INTRODUCTION

Two microorganisms selected for study were leptospires (shed in urine) and salmonellae (shed in feces) of infected animals. In the U.S. these pathogens are widespread and are of major public health and economic importance to both man and animals.

In 1959, 22 states reported a total of 100 cases of human leptospirosis. This figure indicated a 41% increase over the previous year. In 43 of the 100 cases in which possible source of infection was noted, 19 or 44% implicated water as the source (7). As many as 100,000,000 leptospires have been reported shed per ml of cattle urine (8). Infected cattle shed the organisms for periods up to several months. In the U.S., infections in cattle and swine are common. The most common serotype infecting cattle is Leptospira pomona. Leptospires survive for days to weeks outside the live animal. In recent research, leptospires survived up to 138 days in the manure of the field-simulated laboratory model of the oxidation ditch.

As an epidemiologist in field study of leptospirosis, the principle investigator has observed human cases of leptospirosis associated with aerosol-borne transmission from the urine of infected cattle in farmers, veterinarians, packinghouse workers, and hunters. The likely problem of aerosol transmission of leptospirosis from animal manures has not been defined. Infectivity had not been measured.

In the U.S., salmonellosis is one of the major communicable disease problems. There are an estimated 2 million human cases per year. In 1972 there were 22,151 isolations of salmonellae reported from humans. Salmonella typhimurium was the most frequently isolated serotype from both human and nonhuman sources, including cattle (9). A close correlation of the same serotype isolated from human and animal cases was documented. From 1963-1967, S. typhimurium was frequently isolated from individuals involved in epidemics (10). Control of the ubiquitous salmonellae represents a major challenge to the fields of public health, veterinary science, agriculture, and the food industry. Salmonellosis causes substantial losses to the livestock and poultry industry. In recent years, enteritis in dairy and beef cattle caused by salmonellae has increased. Salmonellae are discharged in cattle feces for extended periods of time. In acute cases in calves, 10,000,000 organisms per gram of feces have been reported (11). The cost is estimated at $300,000,000 annually. Salmonellosis is a threat to everyone as a food-borne disease.

Several researchers have demonstrated that salmonella occurs in the upper respiratory tract before onset of gastrointestinal signs and suggest that the transmission of the pathogen might be airborne (12). In food-processing facilities, contaminated air supplies leading to processing facilities have been found to be the direct cause of the finished product contamination.

In experiments utilized to infect mice with mono-dispersed aerosols of Salmonella typhimurium, the lethal dose was very much smaller than required by ingestion and about equal to the intraperitoneal lethal dose (13). In 1957, Moore reported that the conjunctival route was far more effective than the oral route for producing systemic salmonellae infection in guinea pigs (14).

Extreme growth temperature ranges for salmonellae are found between 7° C and 45° C, with a pH range of 4.1-9.0 and optimum pH of 6.5-7.5 (12).

Gibson (15) in a review cites a number of references pertaining to survival. Serotype typhimurium survived 120 days in water and 280 days in garden soil. In England, S. typhimurium survived for 12 weeks when seeded in cattle slurry (16). One project objective was to study the potential transmission of disease-causing agents (pathogens) from cattle manure (solid waste) to animals by the aerosol route. Potential public health effects of pathogens in the environment of animal production units which utilize the oxidation ditch for treatment and disposal of manure wastes were studied. Pathogens excreted by animals may become aerosolized and incorporated as respired air breathed by animal and man alike. The first method of approach was to utilize model situations and laboratory animals. A 1:10 scale model oxidation ditch with isolator animal housing unit was used to simulate the Rosemount Pasveer field oxidation ditch and animal production facility (Figure 1).

A most important consideration was the host-environment-agent (pathogen) interaction in the production of disease. If laboratory animals died or became ill during the study, it was incumbent to determine whether or not disease was produced by the pathogen under study or if there was a cause-effect relationship. The experiments were started with a pathogen of known virulence as determined by pathogenicity in laboratory animal studies. A known dose not exposed to the manure environment should have produced infection in host populations. Whether or not isolates from the manure were altered by

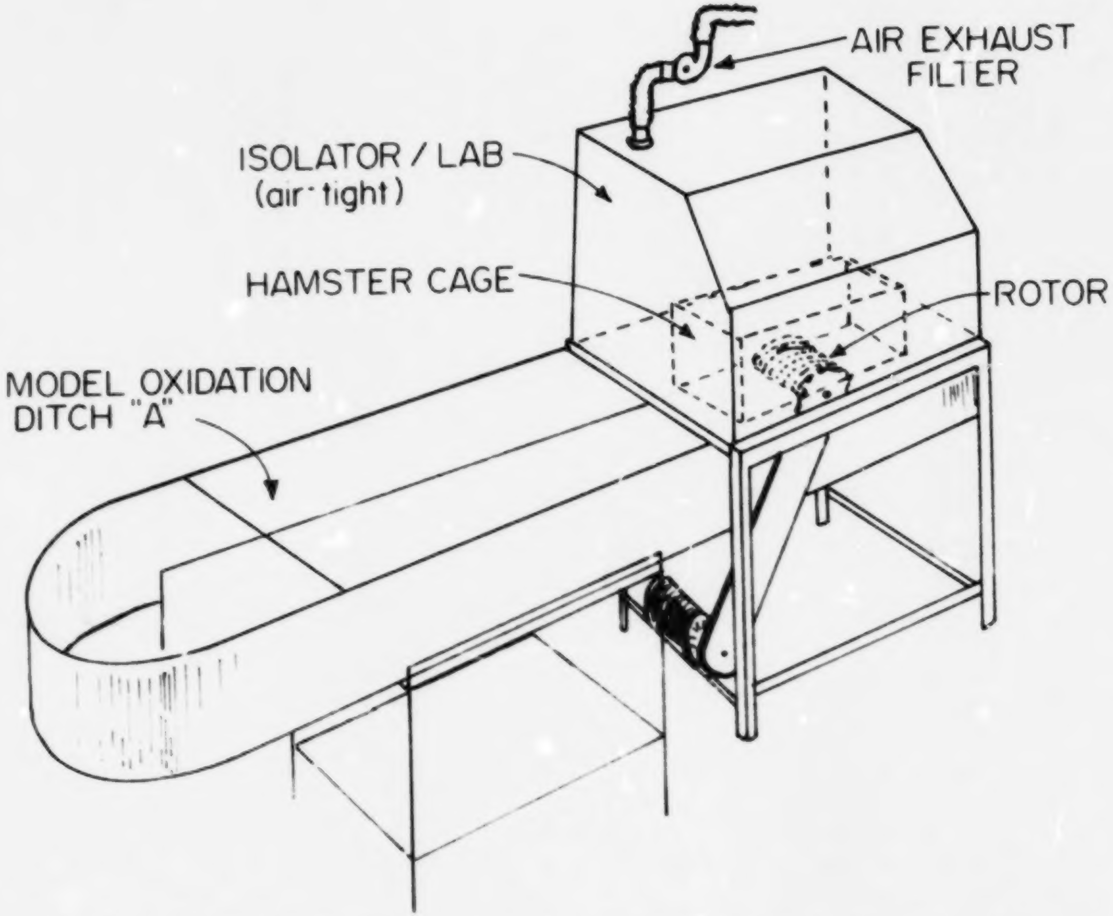
AIR EXHAUST
FILTER
ISOLATOR / LAB
(air-tight)
HAMSTER CAGE
ROTOR
MODEL OXIDATION
DITCH "A"

Figure 1. Schematic of oxidation ditch model with animal isolation facility

the environment to which they were exposed was an intriguing question.

As described herein, effective control and measurement of the host, the environment, and the agent in a simulated situation was accomplished.

General Materials and Methods Protocol Underlying Phase I (Leptospiral/Hamster) and Phase II (Salmonella/Poult) Studies

Susceptible laboratory animals were housed in open-floored units situated above the rotor of a 1:10 scale model oxidation ditch (hood)to simulate beef cattle confinement facilities associated with field Pasveer ditch operations. The MOD (model oxidation ditch) manure slurry was inoculated (seeded) with the selected urinary or fecal tract pathogen. Groups of laboratory animals were exposed for short periods of time (1-4 weeks) to the potentially pathogen-contaminated aerosol and surface. Small groups of laboratory animals were also fed recycled feed (RCF) or exposed to other experimental conditions. If these situations were microbiologically hazardous, the laboratory animals should monitor the extent by expressing disease, infection, or no response as determined serologically through antibody conversion or by isolation of the pathogen.

Four leptospiral and four salmonella experiments were conducted. Two of each were at winter manure slurry temperatures (2° C) and the remainder at summer manure slurry temperatures (20° C). These temperatures had been determined from field data obtained in previous research.

MATERIALS & METHODS

Phase I Leptospiral Studies

Oxidation Ditch, Model A (MOD-A) --

During the first three years (1968-1971) of this research grant entitled "The Survival of Pathogens in Beef Cattle Manure", a 1:10 scale model of the Pasveer field unit located at the University of Minnesota's Rosemount Experiment Station was constructed. For these studies the model (MOD-A) was overhauled and slight modifications were made. Stainless steel rotor and rotor shield were added. Since the original motor/gear burned out during a trial run, the motor and speed-adjusting apparatus were replaced by newer, more durable units. A thermal coil through which coolant circulated was attached to the center divider of the model ditch. Manure slurry temperatures were regulated by altering the temperature of the coolant circulating through this coil. The refrigeration/heating unit was originally located beneath the MOD-A.

The Housing Unit --

A Fisher Isolator/Lab available in the Department of Veterinary Microbiology and Public Health was modified into an animal housing/aerosol study chamber and located over the rotor of the MOD-A. The airtight unit had a Cambridge absolute filter in the exhaust airstream for removal of pathogenic microbes. A stand for this isolator was built so that it could be properly situated atop the model oxidation ditch immediately above the rotor (Figures 1a and 2). A hole was cut from the bottom of the isolator and either a cage capable of housing 20 hamsters or wire mesh flooring for poults was placed within the isolator. Food and water were available at all times in open troughs and waterers. Therefore, the food, water, and environmental surfaces of animals were continuously exposed to aerosol contaminants if present in the ambient air (Figure 3).

Environment Control Room (ECR) --

A portion of a laboratory in the Department of Veterinary Microbiology and Public Health was utilized to construct an environmental control room consisting of interlocking insulated panels. The prefabricated sections were purchased and installed by members of the Department of Agricultural Engineering. This room assured uniform, ambient, experimental temperature conditions. During 1972, installation of the environmental control room was completed. The ECR unit enclosed the Model A oxidation ditch-animal housing unit complex and the instrumentation for MOD-A slurry and environmental data monitoring. Phase I and II experiments utilizing the MOD-A lab animal housing unit were conducted within the ECR with the exception of the winter condition leptospiral studies.

Animal Model (Hamsters) --

A susceptible host to leptospiral infection is the weanling Syrian hamster. Male, weanling (21-day-old, 20-40 grams) hamsters were selected as subjects because of favorable colonizing and compatibility traits. Also, a continuous supply of the Syrian hamster was assured.

During leptospiral experiments, some hamsters quartered in the animal housing unit above the MOD-A died. The cause was usually not

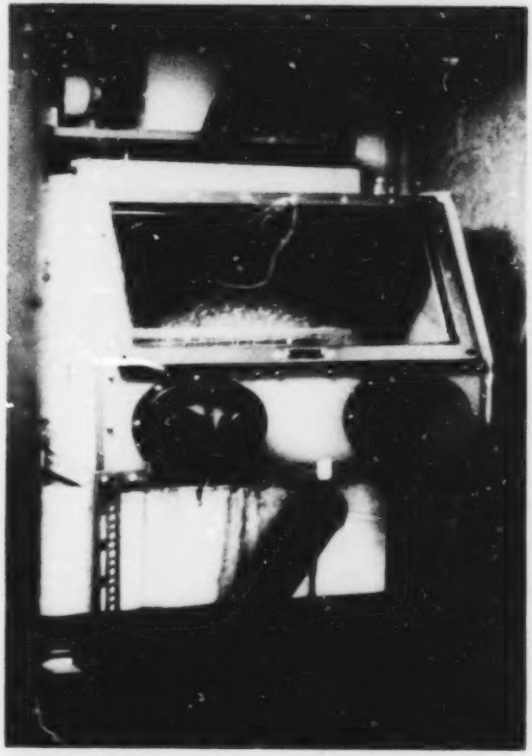

Figure 1a. Model oxidation ditch with animal isolation facility

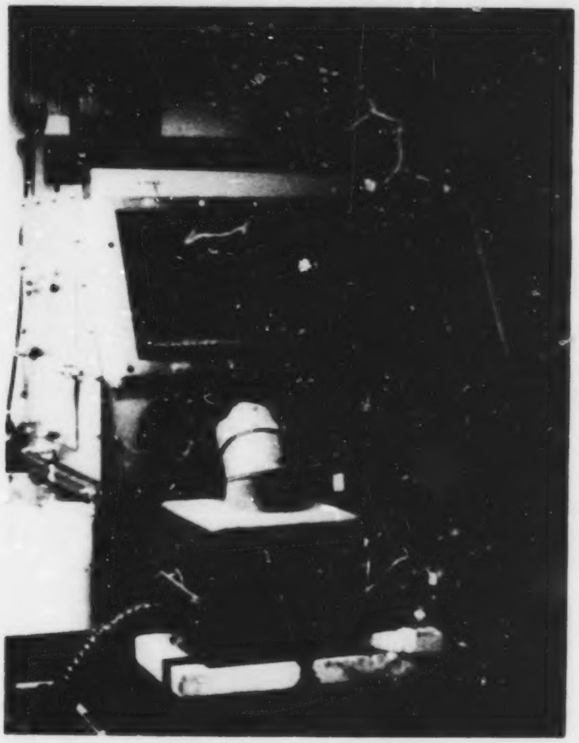

Figure 2. Lundgren Electrostatic Aerosol Precipitator (LEAP) sampling the air Model A

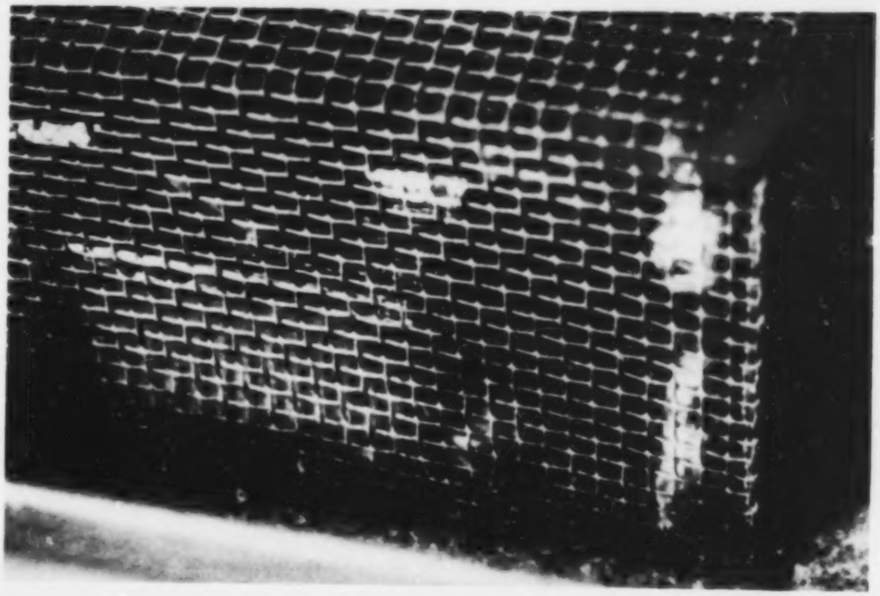

Figure 3. Hamster cage inside the animal housing unit above the Model A oxidation ditch

ascertained since the remaining hamsters rapidly cannibalized their dead or dying mate, usually leaving little or no material to culture. Such specimens salvaged were all found negative for Leptospira sp., indicating that death resulted from battle rather than disease (a "pecking order" is established and weakest hamsters are killed). All such hamsters or hamster deaths were reported as negative results.

Agent, Pathogen --

On January 3, 1972, Leptospira serotype pomona MLS cultures were obtained from Dr. Herman C. Ellinghausen, Jr., National Animal Disease Laboratory (NADL) at Ames, Iowa. Serotype re-confirmation was made by two-way absorption test by the Bacteriology Section of the Center for Disease Control, Atlanta, Georgia. These stock cultures were maintained and used exclusively in Phase I research.

Agent: MOD-A Slurry Isolate --

During experiment first leptospiral, winter conditions, (L.1.W.), isolation of leptospires from the previously seeded MOD-A manure slurry was achieved with the filter-agar plate technique. These isolates were serologically identified by the Center for Disease Control as Leptospira serotype pomona MLS.

Minimum Infectious Dose (MID) Virulence Testing --

MID studies were conducted on stock Leptospira serotype pomona MLS and MOD-A manure slurry isolate cultures. In each study, from 50 to 55 hamsters were used. Five hamsters per dilution/concentration were injected intraperitoneally with 10-fold reductions from 20 x 10^8 to 2 x 10^0 or 2 x 10^1 leptospires and a control group injected I/P with sterile diluent. The quantity of organisms was determined nephelometrically and calibrated with a Roessler N20 standard. Twenty-one days after inoculation, the hamsters were sacrificed, blood was drawn, and kidneys were aseptically removed for culturing. Serum agglutination testing for determination of antibody titer and kidney culture isolation of inoculated hamsters were used to measure infection.

Media --

The basic leptospiral culture medium was Ellinghausen's bovine serum albumen (BSA) polysorbate 80 (17). Variations of this medium were: (a) 1% of BSA diluent: a 200-ml stock bovine serum albumin in phosphate buffer, plus 800 ml of diluent containing phosphate buffers in water; (b) semisolid agar medium as 1% BSA medium with 2.5 g agar for a 0.2% agar/1% BSA concentration and (c) agar plate medium as 1% BSA with 12.5 g agar for a 1% agar/1% BSA concentration. The media were prepared in the laboratory and incubated at 37.5° C, 29° C, and room temperature for 24-hour periods and observed for contamination. Media were stored at room temperature or in a 2° C refrigerator.

Manure and Water --

Beef cattle manure slurry (feces and urine mixture) was collected from the field oxidation ditch at the University of Minnesota, Rosemount Experiment Station. Water was obtained from the Station's well adjacent to the ditch facility. The water and manure were transported to the veterinary microbiology laboratory on the St. Paul Campus of the University where the manure was refrigerated at 0° to 1° C until used. During the start-up of an experiment, these components were mixed in the Model A oxidation ditch in such proportions as to achieve a manure slurry in terms of microbial, chemical, and physical processes, the model ditch was operated for one week prior to inoculation with pathogens. Winter experiments were conducted at 2° C manure temperature; summer studies at 20° C manure temperature. During this period, microbiological sampling was begun.

Monitoring the MOD Manure Slurry Physical State --

The parameters measured routinely in the Model A oxidation ditch manure slurry were: pH, dissolved oxygen (D.O.), temperature, and total solids. Sensing of the MOD-A manure slurry pH, temperature, and dissolved oxygen (D.O.) was conducted by appropriate electrodes and field-laboratory equipment. The electronically measured parameters were recorded by a Beckman 10" strip chart recorder at six-hour intervals.

The ambient air temperatures and relative humidity were continuously recorded on a Serdex Hygrothermograph. Barometric pressure was recorded daily by laboratory personnel from an aneroid barometer.

Vitamin B Analysis of the Manure Slurry --

Duplicate samples of the Model A oxidation ditch manure were submitted to WARF Institute, Madison, Wisconsin, for Thiamine HCL (Vitamin B_1) and Vitamin B_{12} determinations. These two vitamins are essential for optimal leptospiral growth, according to Dr. H. Ellinghausen.

Seeding of the Model Oxidation Ditch-A Slurry --

During the initial stages of each leptospiral experiment, 37.3 billion log phase L. pomona MLS were inoculated into the manure slurry of the Model A oxidation ditch. For each of the four experiments, there were five daily seedings for a total of 1.9×10^{11} leptospires, or 1.7 million/milliliter of manure slurry (Table 1). Thereafter, no leptospires were seeded into this material. A modification of a previously used procedure for preparation of the leptospiral inoculum was made. Rather than resuspending the leptospires to a nephelometer reading of 25 (Roessler Standard: N=20) which required a large amount (373 ml) of diluent, the leptospiral organisms were resuspended after centrifugation with the minimum amount of diluent which would give a nephelometer reading. The number of organisms was determined nephelometrically from this suspension, the total quantity of which was generally about 75 ml, or 1/3 to 1/4 of the amount previously utilized in seeding the MOD-A slurry. This procedure was considered more accurate because a direct nephelometer reading was made on the inoculum, whereas we previously depended upon proper dilution after nephelometric determination to quantify the inoculum.

Sampling, MOD-A Manure Slurry --

Pipettes were used to aspirate manure slurry samples from MOD-A at six sites, three effluent (top) and three sludge (bottom), (Figure 4). Two methods of culturing for leptospires from these slurry samples were incorporated during the studies. Only the agar plate technique was utilized throughout all four leptospiral experiments.

In the tube dilution technique, approximately one milliliter of manure was pipetted into a tube of 1% BSA diluent. Ten-fold serial dilutions and transfer to Ellinghausen's BSA was made of this material which was then incubated at 29° C and observed periodically for 10 weeks by darkfield microscopy for the presence of leptospires. This procedure was not efficient for isloation of leptospires from highly contaminated material such as the manure used in these experiments.

In the agar plate technique, six drops of manure slurry were placed in the center of a Millipore, .22-μ pore size, type GS filter which was on the surface of BSA agar plate. The leptospires migrated through this filter to establish growth in the agar medium. These plates were incubated at 29° C and examined for evidence of cultural detection of leptospiral survival and has allowed continuous

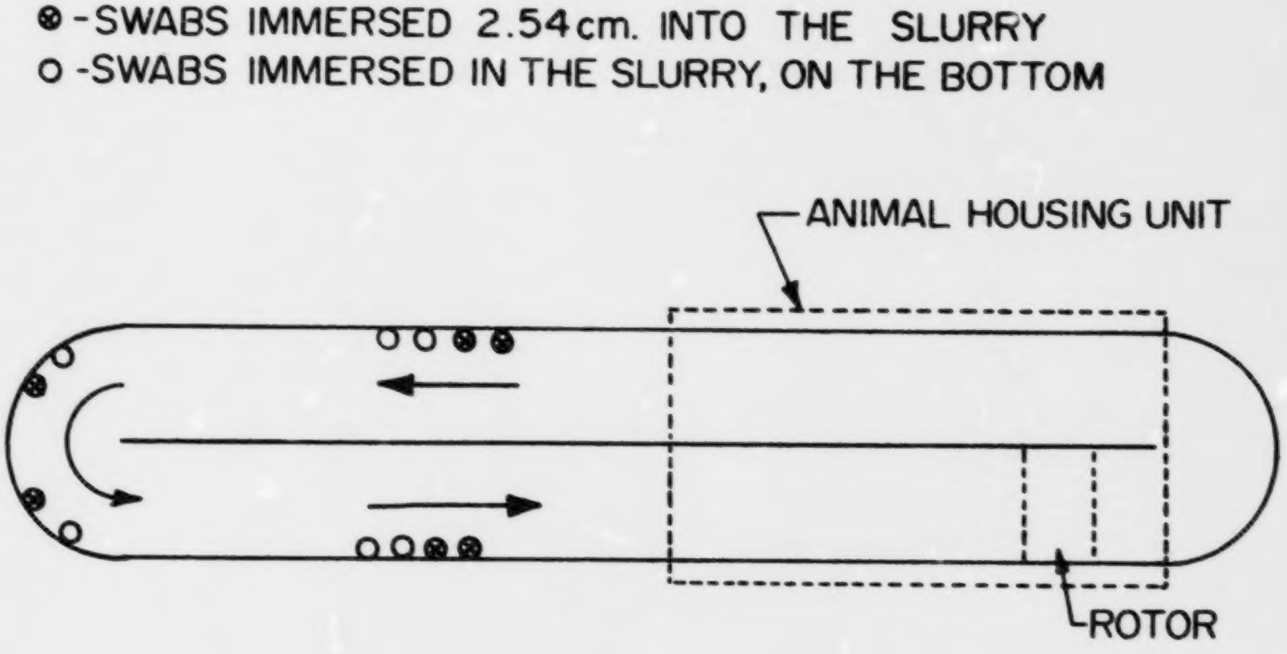
⊗ -SWABS IMMERSED 2.54cm. INTO THE SLURRY
○ -SWABS IMMERSED IN THE SLURRY, ON THE BOTTOM
ANIMAL HOUSING UNIT
ROTOR

Figure 4. Sampling sites of model oxidation ditch-A

Table 1. INOCULATION OF MOD MANURE SLURRY WITH LEPTOSPIRES

Experiment Number	Date of Inoculation in 1972
L.1. Winter	March 7 March 8 March 9 March 10 March 13
L.1. Winter	May 30 May 31 June 1 June 2 June 3
L.3. Summer	July 26 July 27 July 28 July 31 August 1
L.3. Summer	October 10 October 11 October 12 October 13 October 14

leptospiral detection in the manure slurry until the termination of each experiment. Sampling the manure of the model oxidation ditch was conducted three times a week (Monday, Wednesday, Friday).

Aerosol of (MOD-A) laboratory animal housing unit --

A Lundgren Electostatic Aerosol Precipitator (LEAP) was selected for sampling the ambient air of the model housing facility in preference to the All Glass Impinger (AGI) which had been proposed. This choice was reasonable since the LEAP samples up to 1,000 liters of air/minute as opposed to 12.5 liters/minute by the AGI. Also, leptospires were detected in samples collected with the LEAP but not by the AGI in a pre-experiment study (Table 2). This experiment was conducted in an isolator unit in our laboratory not associated with the

Table 2. DETECTION OF L. POMONA MLS IN AEROSOLS

Method of Detection	Experiment Number 1	2	3	4	5
1% BSA Liquid Media	-	-	-	-	-
1% BSA Agar Plates	-	-	-	-	-
Lundgren Electrostatic Air Precipitator	+	-	-	+	-
Hamster (Serum agglutination)	-	-	-	-	-
Kidney Isolation	-	-	-	-	-

Model A oxidation ditch. An attempt was made to saturate an enclosed environment with an aerosol of L. pomona MLS. Within the isolator were located the following:

1. one nebulizer for production of homogeneous air dispersion of L. pomona MLS.
2. two AGI impingers for sampling the air.
3. five liquid BSA media in petri dishes.
4. five agar BSA plates.
5. five male weanling hamsters.
6. A LEAP mechanism capable of sampling the entire air content of the isolator was attached to the unit.

Furthermore, because detection was essentially by darkfield microscopy, the larger volume of air and, thus, the concomitantly greater bioload sampled by the LEAP provided greater sensitivity than the AGI. The fluorescent antibody technique (FAT) was not utilized for these studies. The FAT is a time-consuming detection method when compared to the filter-agar plate technique which employs darkfield microscopy successfully for detection of viable leptospires whereas the FAT does not indicate viability.

Utilizing the same arrangement as described above with the exception of AGI and liquid medium plates, another study was conducted with the L. pomona MLS on March 21, 1973.

Effect of MOD-A Rotor Speed upon Aerosol Production --

On November 16 and 17, 1972, a series of aerosol samples were collected from the MOD-housing unit with the Lundgren Electrostatic Aerosol Precipitator (LEAP) and AGI. Each sample was collected at various ditch rotor speeds designated as "slow" (normal operating speed), "fast", and "fastest." The normal operating rotor speed during all of the experiments was determined empirically as that rotor speed which came closest to propelling the manure slurry at 45.72 cm/second which was not at the greatest rotor speed, but that designated "normal" which was a relatively slow speed.

Refeeding Experiments --

In order to study the effects of recycling pathogen-contaminated manure to animals, refeeding experiments were conducted with hamsters. Reclaimed solid wastes from the Rosemount pilot field oxidation ditch, mainly corn, was utilized in our studies. Three hundred cubic centimeter volumes of recycled corn were wrapped in double-layer, 4-thickness cheese cloth which was closed, tied, and pressed to eliminate excess liquid. Thus prepared, the recycled feed (RCF) was frozen and stored at 0° C for use as required. One sack of RCF was suspended in the stream of the model oxidation ditch for successive one-week periods, after which time the leptospiral-contaminated content was fed to a new group of five male weanling hamsters not housed in the unit. Following consumption of the RCF (usually one day), these hamsters were fed commercial hamster feed.

Various Routes of Inoculation Studies --

Since laboratory and domestic animals are exposed by various routes while held in confinement facilities, testing of other than respiratory routes of infection was necessary. Therefore, experiments were conducted utilizing hamsters. One droplet of a suspension of L. pomona MLS was inoculated topically onto nares, oral cavity, or conjunctival surface. The suspensions were estimated to contain from 1×10^3 to 2×10^8 L. pomona MLS per droplet.

Infection was measured by both the microscope agglutination (MA) test of serum and by kidney culture techniques.

Three variable route-of-inoculation studies utilizing L. pomona MLS were conducted on male weanling hamsters to determine either infectiousness or the minimum dose required to cause infection. In one experiment, pelleted rat chow (feed) was inoculated with pathogenic leptospires and fed to susceptible hamsters.

PHASE II, Salmonellae Studies

During the period of November 15, 1972, to December 15, 1972, the MOD-A and animal housing unit was overhauled and refitted to accommodate week-old turkey poults. A poultry feeder, water trough, brooder heater, and light were installed within the housing unit. The gear unit on the motor was rebuilt as was the ditch rotor and the rotor mount. The cooling system was modified to exclude coolant circulation through a diffuser in the environmental control room since warm, ambient air conditions were required for this age poult as opposed to the cool temperatures preferred for hamsters. The system continued to circulate coolant through the MOD-A to maintain and regulate slurry temperature.

The change-over from hamsters to poults created several difficulties which required time and experimentation to rectify. Young poults were very susceptible to environmental stress; thus both morbidity and mortality were required to obtain results and additional samples were necessarily required because of the increased number of animals, and more samples were taken from each bird. More labor and media were required to follow a specimen through the salmonella culture procedures than was the case for leptospiral detection and identification.

Procedural Protocol --

Thirty-five poults, one-day-old, were purchased each week and grown for approximately seven days to "week-old" poult stage. At one week of age, this group was ideally to be separated into the following categories:

Number Proposed: Week-old Poults	Use
20	Housed over MOD
7	Fed contaminated "starter" feed
5	Control
3	Expected "normal" mortality (10%)

The actual numbers in the Salmonella poult experiments ranged as follows:

Number Utilized: Week-old Poults	Use
3-24	Housed over MOD
3-10	Fed contaminated starter
0-10	As controls
0-18	Mortality

Each salmonella experiment was conducted for a duration of about five weeks: one initial week for stabilization of the MOD-A, five days for seeding the manure slurry, and a final thirty days for experiment run-time (Table 3).

One obstacle which was not overcome concerned the timing interval for purchase of poults. Each week, 35 day-old poults were purchased from the second largest turkey hatchery in Minnesota. This producer could not assure an evenly spaced interval between poult hatches. The supply problem necessitated fluctuating poult ages and exposure duration which potentially complicated statistical evaluation.

Poults --

Discussions were held to decide whether mice or poults should be the laboratory animal model of choice for the salmonella studies. The grant proposal was designed for poults but some practical considerations, such as their size with increasing age, comparative susceptibility to the agent, freedom from congenital or neonatal salmonellosis, and seasonal supply caused concern whether they would be suitable. Poults were selected.

A literature search disclosed that susceptible poults over 2 to 3 days of age would become infected with salmonellae, but changes of fatal infection greatly diminished after this age. With this knowledge, the best sites for cultural isolation of salmonella from the poults were determined. Routine sampling was conducted of the liver, caecal junction, and oral cavity on all experimental poults. Once the poults were dead or sacrificed, tongue, liver, or caecal tissue was dissected from the poult, alcohol drenched, flamed, cut up, and placed into Selenite Brilliant Green Sulfadiazene (SBGS) and followed through as described.

Day-old poults were either purchased from Moorhouse Turkey Hatchery, Inc. or obtained from Dale Peterson Hatchery when Moorhouse could

Table 3. INOCULATION OF MOD MANURE SLURRY WITH SALMONELLA

Experiment Number	Date of Inoculation in 1973
S.1. Winter	January 9 January 10 January 11 January 12 January 15
S.2. Winter	March 20 April 16 April 17 April 19
S.3. Summer	June 17 June 19 June 20 June 22 June 23 July 10
S.4. Summer	August 7 August 9 August 10 August 13 August 14 August 24
Restart:	September 5 September 7 September 10 September 13

not supply the required number. Cloacal swabs were taken from all 35 week-old poults of that week's hatch before subjecting them to experimental study.

Salmonella typhimurium stock cultures used in Phase II had been stored on trypticase soy agar (T-Soy) slants and transferred at 3-6 month intervals. Throughout the five years of study, this cultural line was confirmed repeatedly both biochemically and serologically as being somatic antigen: Group B, 1, 4, 5, 12, and flagellar antigen: Phase 1 (i) and Phase 2 (1 complex).

Salmonella Sampling --

The general protocol for isolation of Salmonella has been: sample → Selenite Brilliant Green Sulfadiazene Broth (SBGS) and Brilliant Green Bile Broth (BGB) 37° C/24 hrs → Brilliant Green Sulfadiazole (BGS) Plates and Xylose Lysine Desoxycholate (XLD) Plates 37° C/24 hrs → fish colonies to Triple Sugar Iron (TSI) agar slants 37° C/24 hrs → biochemistry (dulcitol, salicine, urease, lysine decarboxylase → serotyping. Specimens or samples from other than the manure slurry follow the same flow scheme except that only SBGS enrichment and only one of the selective plates are utilized.

It is interesting to note that during the first three years of this project, prior to incorporation of poults to the experiments, BGB was the most successful enrichment medium. Thus far, less than one-tenth of the Salmonella positive samples were grown in BGB. The possibility that the medium was faulty was investigated, but also consider that extraneous enteric microorganisms contributed by the poults is competitive with and selected in preference to the salmonella in this medium.

Minimal Infectious Dose --

Four MID experiments were conducted to quantitate the virulence of the *S. typhimurium* which was being maintained on T-soy agar slants. These were transferred to GN (Hadjna) broth, incubated at 37° C for various periods of time, and diluted. These culture dilutions were plated on Brilliant Green Sulfadiazine (BGS) agar and a 1-cc aliquot of each dilution was injected into test birds. Plate counts gave a measurement of the quantities injected. The broth cultures were not sonicated or shaken to break colony clumps into individual bacteria (Table 4).

Manure and Water --

MOD-A manure slurry was that beef cattle manure slurry obtained from the Rosemount FOD plus added well water from Rosemount for a proposed initial total solids content of 5,000 to 10,000 mg/l at the start of each MOD-A study (Table 5 indicates the actual initial total solid values). All manure and water were cultured for Salmonella or Leptospira, depending on the experiment prior to use in the MOD-A.

Table 4. DETERMINATION OF MINIMUM INFECTIOUS DOSE (MID) OF STOCK SALMONELLA TYPHIMURIUM INOCULATED INTRA-PERITONEALLY (I/P)

Experiment No. 1 (I-2)

Group Number	Concentration in Inoculum Administered	Initial # of Poults	Number Died	Number Positive
1	17×10^4	10	1	7
2	17×10^6	19	3	14
3	17×10^{10}	19	0	18
4	17×10^{14}	20	4	20
5	17×10^{17}	21	16	21
Control		11	0	0

All poults were cloacal swab culture negative for S. typhimurium.

Experiment No. 2 (I-2)

Group No.	No. of Poults	Dose I/P	Post-Mortem No. Positive*	Anti-Mortem Positive (Oral)/ Population
1	6	98×10^2	1 oral	1 Oral 1/6 Liver 0/6 Caecal 0/6
2	9	98×10^5	8	3 Oral 7/9 Liver 5/9 Caecal 6/9
3	4	98×10^{11}	4	4 Oral 4/4 Liver 3/4 Caecal 3/4
Control	8	0, Broth Only	0	0 All 0/8

*Organs

All poults were cloacal swab culture negative for S. typhimurium.
1.0 cc inoculum I/P.

Table 4. (continued) DETERMINATION OF MINIMUM INFECTIOUS DOSE (MID) OF STOCK SALMONELLA TYPHIMURIUM INOCULATED INTRA-PERITONEALLY (I/P)

Experiment No. 3 (I-3)

Group No.	No. of Poults	Dose I/P		No. Positive
1	10	80×10^{18}	10	Oral 10/10 Liver 5/10 Caecel 9/10
2	8	80×10^{16}	8	Oral 8/8 Liver 5/8 Caecel 6/8

All birds were positive orally antimortem. Cloacal swabs negative. 1 cc injected I/P.

Experiment No. 4 (I-4)

Group Number	No. of Poults	Number *S. typhimurium*	Culture Sites	Death
1	15	21×10^2	3 1 2	0
2	14	21×10^3	1 2 1	1
3	12	21×10^4	1 0 1	0
4	12	21×10^5	2 2 5	0
5	12	21×10^6	5 5 5	0
7 Control birds - all negative				

All cloacal swabs negative. 1 cc injected I/P.

Monitoring --

The MOD-A manure slurry was monitored for pH, D.O., and temperature as described in Phase I.

Table 5. TOTAL SOLIDS: EXAMPLES OF BUILD-UP

Date		Total Solids (mg/l)
Period Prior to and Including 2 weeks of L.l.W.	1-14-72	4,567
	1-28-72	5,466
	3-3-72	15,868
	3-27-72	18,853
Control Period to and Including 1 Week of S.l.W.	12-7-72	8,211
	1-3-73	9,499
	1-17-73	12,017

Seeding --

A loop of *S. typhimurium* stock culture was removed from the T-Soy slant to GN (Hadjna) broth which was incubated at 37° C for 8 to 24 hours. The broth culture was adjusted to 30 nephelometer units on a Nephlo-colorimeter using a Roessler 20N standard as reference. This material was then used for seeding by placing the required amount of culture in 75 ml of buffered saline, then pouring into the MOD-A manure slurry.

Sampling --

During sampling of the MOD-A for *Salmonella* early in the first Salmonella experiment, Calgiswab (tipped applicators) were taped to the sides of the MOD-A channel walls with the tips of six swabs submerged in the effluent (top-most 2.54 cm of manure slurry) and six in the sludge portion (bottom-most 2.54 cm). Thus, there were two swabs at each of six sites, three effluent and three sludge, as indicated in Figure 4. These swabs dissolved in broth culture as the result of chemical and microbial action. Thus, sampling began on the MOD-A slurry at the prescribed positions with medicine droppers. The dropper sample was 1-5 ml of manure slurry.

Microbioaerosol Sampling of the MOD-A Animal Housing Unit --

Sampling of the MOD-A poultry house air was conducted. Total bacterial count, fecal streptococci, coliform, and salmonella determinations were made of the microbial profile as described in Section V, Field Aerosol Studies. The Lundgren Electrostatic Aerosol Precipitator (LEAP), sampling 1,000 liters of MOD-A poultry housing air per minute, was utilized throughout these studies. The poultry housing unit had an air volume of 11.1 cubic feet or 315.2 liters. Theoretically, the LEAP sampled the ambient poultry air approximately three times every minute. The collecting fluid used was 0.1% peptone. The samples were analyzed for total coliform, fecal streptococci, and salmonella organisms.

The LEAP sampled airborne particles were deposited onto a thin, moving film of liquid collecting medium. Air entered the sampler through a nozzle that was calibrated for airflow rate up to 1,000 liters of air per minute. It then flowed over a rotating disc to which is applied a high voltage. The air was then drawn radially through this high potential, up to 20 kilowatts across the plate to disc spacing, precipitating particles onto the collection disc. Collecting liquid was supplied by a pump to the center of the collection disc. Centrifugal force caused the liquid to flow in a thin, continuous film outward across the disc to a pickup dish where the particles were washed. The sample liquid was pumped from the the pickup dish to a container. From the LEAP samples taken at the MOD-A, 0.1 ml of properly diluted collecting fluid was placed on a plate count agar (PCA) and incubated for 48 hours at 37° C. All colonies were then counted.

Likewise, 0.1 ml of collecting fluid from the LEAP sample was placed on Levine Eosin Methylen Blue Agar (L-EMB) and colonies that were large, pigmented, gram negative rods, and fermented lactose with the production of gas on TSI were counted.

Fecal streptococci determination was made by plating collecting fluid on M-Enterococcus agar (M-ENT). Characteristic red colonies produced on M-ENT were also tested for catalase negativity and the production of a black color on Bile Eschelin Agar (BEA).

The presence or absence of salmonella was analyzed in samples taken with the LEAP by placing 2 ml of collecting medium into enrichment broth of Selenite Brilliant Green Sulfadiazene (BGS) and incubating at 37° C for approximately 24-28 hours. From each broth solution a loopful was streaked onto Brilliant Green Sulfadiazene (BGS) agar and Xylose Lysine Desoxycholate (XLD) agar and incubated for 48 hours at 37° C. The characteristic reactions were classified as salmonella. In addition, 0.1 ml of collecting fluid was placed directly onto BGS and XLD agar to further check for the presence of salmonella.

Aerosol experiments --

Initially, two experiments were conducted to test aerosol production and microbioaerosol sampling ability. Later, five experiments were conducted to ascertain quantitatively the infectivity of Salmonella typhimurium aerosols. These experiments were conducted in an isolation box identical to that converted for the MOD-A animal housing unit complex. The salmonella broth cultures were nebulized with a DeVilbiss Model 640 Clinical Glass Nebulizor. The aerosol thus produced was sampled both by the LEAP connected to the isolator by a galvanized metal 90° elbow and by open petri dishes (fall plates) containing selective media (XLD or BGS) placed on the floor of the isolator or on top of the cage within which the poults may or may not have been placed.

During some of the aerosol experiments as well as other experiments described below, the tongue-pharyngeal region was investigated as a possible sampling site for the detection of salmonella infection.

Route of Inoculation --

A study was conducted with 54 poults to determine oral, ocular, and nasal routes of susceptibility to S. typhimurium infection. Culture suspensions were placed by dropper onto these sites and streaked onto BGS plates for quantitation.

Recycled Feed (RCF) --

Two types of studies were conducted to determine the effect of feeding pathogen-contaminated RCF to poults. One series of experiments was conducted as part of the four MOD-A experiments. Poult "starter" feed wrapped in cheesecloth was submerged in the manure slurry of the MOD-A for varying periods of time (one to several days), after which it was cultured for salmonella and fed to week-old poults. The design of this series was an attempt to simulate the feeding to domestic animals of manure slurry solids obtained from field oxidation ditches. This feeding practice is currently occurring to some extent at universities and in private animal industry throughout the U.S. Initially during control experimentation, the slurry solids from the Rosemount oxidation ditch were fed to the poults as had been done with hamsters during Phase I research. The poults did not accept this material, however, and the "starter" ration was substituted as an alternative to the recycled feed.

In another experiment, 120 one-week-old poults in groups of eight were fed 2.86 kgs. of "starter" ration which was contaminated with 20 ml of various concentrations of S. typhimurium. After the contaminated starter feed had been consumed, the poults were fed non-contaminated starter. All poults were sacrificed and cultured one week after being fed the salmonella-contaminated rations.

Oral dosing studies were also conducted. Eight week-old poults were placed in each of two groups. Two poults from each group were sacrificed on each of the first four days. Each group had been orally inoculated with a different dose of S. typhimurium (154 x 10^7 and 154 x 10^2).

RESULTS AND DISCUSSION

The Phase I and II endeavor throughout the two-year period of Grant No. R802205 was to study selected microbial pathogens and hosts in association with an operational model oxidation ditch (MOD). The conditions created for study were "practical worst care" situations, that is, more severe conditions could have been established but those created in the laboratory were similar to those expected in the worst cases described in field situations. The MOD was a 1:10 scale model of a field unit. The winter (2° C) and summer (20° C) MOD slurry temperatures were derived from data obtained from the field unit. Likewise, the total solids (T.S.) parameter was determined from provious knowledge of oxidation ditch operation and function. Most important perhaps, was the determination of the quantity of pathogen to be added to the MOD manure slurry which was based upon known excretion rates. Calves may shed 10,000,000 salmonella per gram of feces, and up to 100,000,000 leptospires per ml are excreted in urine of infected, shedding animals.

A total of 1 x 10^{11} Salmonella typhimurium were seeded at the beginning of each salmonella experiment resulting in a concentration of 8.7 x 10^5 organisms/ml of MOD manure slurry if a reduction of microorganisms did not occur. However, there was a decimal reduction rate of S. typhimurium in the slurry as evidenced by finite survival duration which varied with MOD conditions (6). Furthermore, the figures utilized are, at best, approximations which may flucuate by an undetermined amount. The best estimation would be ± 50% at the time of nephelometer determination since the microorganisms are not necessarily monodispersed in solution but, rather, may form aggregates. The cultural measure used to calibrate the nephelometer may lack accuracy. It detects colony-forming units in addition to individual organisms.

The same consideration was true also for *Leptospira* serotype *pomona* MLS seeding. There is a tendency toward clumping in cultural media. After the initial seedings, 1.7×10^6 *L. pomona* MLS were expected (calculated) per milliliter of manure slurry. A decimal reduction time existed for this pathogen in manure slurry, also based on survival time.

Cultural monitoring for pathogens, salmonella or leptospira, gave the only indication of the presence of either. The critical factor for determining the microbiological health hazard associated with the MOD was the presence or absence of pathogen, and since the quantitation of seed numbers only verified that conditions were "worst practical," it was necessary to reseed the slurry whenever the microorganisms could no longer be detected by methods utilized.

The manure slurry physical-chemical data summary for each experiment is given in Table 6. The total solids increased significantly during each experiment, as illustrated by examples in Table 5. The alteration of the MOD manure slurry environment was obviously unavoidable, but similar to real case situations. Survival of *S. typhimurium* and/or *L. pomona* MLS may be decreased as compared to previous research conducted without the continuous introduction of extraneous organic material. However, detection of survival may have been decreased also.

The detection of either *L. pomona* MLS or *S. typhimurium* was not nor could it have been absolute. The methods for cultural detection of microbiological pathogens must be altered with each situation until a best method(s) is developed. Negative results regarding the manure slurry, feed animals, and aerosol to which cultural detection methods were applied in this research cannot necessarily be considered such in the absence of positive results (Table 7). On at least one occasion during each of the four experiments (5.1.W., week 3; S.2.W, week 6; S.3.S., weeks 4 and 5; S.4.S week 6), poults became infected with *S. typhimurium* despite lack of cultural evidence of the pathogen's presence in the RCF (Table 8). In fact, the probability of culturing *S. typhimurium* from the starter feed was low. A study was conducted to determine what concentration of *S. typhimurium* in poult starter feed was necessary for poult infection and/or cultural detection (Table 9). Reasons for the negative results are conjectural: rapid die-off of the microorganisms in the feed; absorption of the microorganism into the feed where it becomes isolated from the culture media; or interaction of feed and media to either neutralize growth/

Table 6. MOD PHYSICAL-CHEMICAL MONITORING SUMMARIES

Exp.	Total Solids (mg/l)	D.O. (mg/l)		Temperature (°C)		pH	
		$\bar{x}$;	range	$\bar{x}$;	range	$\bar{x}$;	range
L.1.W.	5,466	0.5	1.4-01.4	02.0	02.0-02.0	6.5	6.3-6.7
2.W.	5.655	4.4	0.0-06.8	02.0	04.0-08.0	7.2	6.4-7.6
3.S.	4,688	2.6	0.0-10.2	20.0	18.0-27.0	6.3	4.5-7.7
4.S.	13,723	1.1	0.0-06.0	19.8	15.0-27.0	6.4	5.0-6.9
S.1.W.	9,499	4.4	0.0-24.0	03.9	00.0-09.0	7.2	6.0-8.1
2.W.	10,754	2.5	0.0-09.8	01.4	00.0-06.0	7.2	5.8-7.7
3.S.	8,277	Probe not Functional		16.9	14.0-21.0	7.7	5.1-8.4
4.S.	19,287	0.1	0.0-01.0	20.7	16.0-30.0	7.2	5.8-8.0
	6,560	2nd Seeding					

reproduction or produce a toxic substance deleterious to growth and detection.

In those instances where the RCF was culturally negative but poults became infected, correlation was made with the MOD-A manure slurry cultural results for S. typhimurium. If the slurry was positive for S. typhimurium, it is assumed the presence of the pathogen was identified but transmission to the poult was not measured or may not have occurred.

An alternative is to suspect that the poults themselves may have harbored S. typhimurium and expressed infection when stressed during the conditions of the studies. Throughout the course of the research with poults occasionally salmonella would be detected on pre-commitment to experiment by cloacal sample culture. These instances disclosed Arizonae spp. or non-S. typhimurium salmonellae. Therefore, whenever S. typhimurium was detected in a poult, it had been introduced to the poult by the experimental procedure through feeding, exposure to aerosol, or inoculation by other routes.

Although this research was proposed to test the health hazard associated with the potential aerosolization of pathogens from an oxidation ditch (Pasveer) system, it was recognized that animals reared in the area of such a facility would be exposed to more than simply the respiratory route for infection. Microbioaerosols, if produced by the operation of an oxidation ditch, potentially could settle on

Table 7. ANIMAL EXPOSURE, MANURE SLURRY, AND AEROSOL SAMPLING RESULTS OF SALMONELLA/POULT STUDIES UTILIZING MODEL OXIDATION DITCH-A

Experiment	Control	1	2	3	4	5	6	7	8	Total
Slurry	-	+	+	+	+	-	+	-	ND	
S.1.W.										
O. Ditch	0/19	0/8	0/11	1/3	0/20	0/20	0/19	0/19	ND	1/119
Check	0/0	0/2	0/3	0/1	0/5	0/8	0/6	0/7	ND	0/41
Aerosol	-	-	-	-	ND	ND	-	-	ND	
Slurry	-	+	+	+	+	+	+	+	-	
S.2.S.										
O. Ditch	0/15	0/20	0/20	0/20	0/19	0/20	5/21	5/18	0/10	20/163
Check	0/6	0/7	NA	0/4	0/5	1/4	0/5	0/4	0/4	1/39
Aerosol	-	ND	+	-	-	-	-	+	-	
Slurry	-	+	+	+	+	+	+	ND	ND	
S.3.S.										
O. Ditch	Poults	7/15	1/19	1/13	0/15	3/20	0/20	ND	ND	12/102
Check	Not Avail	0/7	0/6	0/3	1/10	0/5	0/6	ND	ND	1/37
Aerosol	-	-	-	+	-	ND	ND	ND	ND	
Slurry	-	+	+	+	+	+	+	ND	ND	
S.4.S.										
O. Ditch	0/20	0/18	0/20	0/19	0/24	0/17	0/16	ND	ND	0/134
Check	0/8	0/5	0/6	0/5	0/5	0/3	0/8	ND	ND	0/40
Aerosol	-	-	-	-	-	-	-	ND	ND	

Table 8. SALMONELLA ANIMAL FEEDING AND RECYCLED FEED SAMPLE RESULTS

Experiment	Control	1	2	3	4	5	6	7	8	Total
S.1.W.										
Feed	ND	ND	0/7	2/4	0/7	0/6	0/8	0/8	-	2/40
Check	0/9	0/2	0/3	0/1	0/5	0/8	0/6	0/7	ND	
RCF		(AP)		(AP)			(AP)			
		0	+	-	-	-	-	-	ND	
S.2.W.										
Feed	ND	0/7	NA	0/8	0/10	0/8	3/8	2/6	10/10	15/57
Check	0/6	0/7	NA	0/4	0/5	1/4	0/5	0/4	0/4	
RCF		(AP)	(AP)		(AP)		(AP)			
		0	0	+	-	+	-	+	+	
S.3.S.										
Feed	ND	0/5	0/6	2/8	1/10	1/10	0/9	ND	ND	4/48
Check	Poults NA	0/7	0/6	0/3	1/10	0/5	0/6	ND	ND	
RCF		(AP)[a]			(AP)	(AP)				
		0	+	+	-	-	-	ND	ND	
S.4.S.										
Feed	ND	0/6	0/7	0/19	0/6	0/7	4/8	ND	ND	4/53
Check	0/8	0/5	0/6	0/5	0/5	0/3	0/8	ND	ND	
RCF		(AP)	(AP)			(AP)	(AP)	ND	ND	
		-	-	+	-	-	-			

[a] AP indicates aerosol positive for salmonella.

Table 9. FEEDING SALMONELLA TYPHIMURIUM CONTAMINATED STARTER TO POULTS

Experiment No. 1 (F-1)

Group	No. S. typhimurium	Results		
		Liver	Caecal	Cloacal
1	25.5×10^{0}	0/8	0/8	0/8
2	25.5×10^{4}	0/8	0/8	0/8
3	25.5×10^{8}	0/8	0/8	0/8
4	25.5×10^{12}	0/8	0/8	0/8
5	25.5×10^{16}	0/8	0/8	0/8

2.86 kgs of poultry starter plus 20 ml of a known concentration of S. typhimurium were mixed in a plastic bag.

feed, water, and other exposed surfaces in the ambient environment; that could contaminate wounds or ocular, oral, and/or nasal membranes. Collateral research was conducted during the grant period to study whether or not infection with L. pomona MLS or S. typhimurium could be induced by pathogen contamination of feed, or eyes, nose, and/or mouth and, if so, at what concentrations or dosage. Kidney culture and/or serologic evidence of infection was induced in male weanling Syrian hamsters by L. pomona MLS when applied to eyes, nose, or mouth but not in feed (Tables 10, 11, and 12). Efforts to infect hamsters when exposed to virulent aerosols of leptospires failed (Table 13). Week-old poults became infected with S. typhimurium when applied to these three sites (Table 14) but not when fed to the poults on feed (Table 9).

The higher dose rate was the only one to consistently induce infection in the poults. Comparison of these oral dosing rates with the contaminated RCF dose rates and the route-of-inoculation rates indicates that the RCF-S. typhimurium dosages were above and below the oral infectious dose in the oral dosing experiment. However, death rate of the salmonella in this situation is not known and some factor may have played a role causing the nil infection rate.

Table 10. *LEPTOSPIRA* SEROTYPE *POMONA* MLS: INFECTION VIA VARIABLE ROUTES OF INOCULATION

Experiment	No. of Leptospires	Positive/Possible			
		Ocular	Nasal	Oral	Feed
1	1.1×10^6	1/4	3/3	1/3	ND
2	1.0×10^3	0/3	0/3	0/3	0/3
	1.0×10^4	0/3	0/3	0/3	0/3
	1.0×10^5	0/3	1/3	0/3	0/3
	1.0×10^6	0/3	2/3	0/3	0/3
	1.0×10^7	0/3	3/3	0/3	0/3
	Control	0/3	0/3	0/3	0/3
Totals		1/22	0/21	1/21	0/18

Table 11. INFECTION OF HAMSTERS BY VARIOUS ROUTES OF INOCULATION

Route	No. of Leptospires	MA				Cultural			
		1	2	3	4	1	2	3	4
Nasal	1.1×10^6	+	+	+	0	+	+	+	0
Ocular	1.1×10^6	+	-	-	-	+	-	-	-
Oral	1.1×10^6	+	-	-	0	+	-	-	0
Control	Saline	-	-	-	-	-	-	-	-

Table 12. LEPTOSPIRAL INFECTION OF HAMSTERS BY VARIOUS ROUTES OF INOCULATION

No. of Leptospires	Positive/Possible		
	Ocular	Nasal	Oral
2×10^4	0/3	0/4	0/3
2×10^6	0/4	0/3	0/4
2×10^8	0/4	0/4	0/4
Control - BSA	---	0/5	---

Another set of experiments was conducted. Poults were inoculated orally and I/P. The oral dose rate was 111×10^2 to 111×10^7; the I/P rate was 130×10^3 to 130×10^{11}.

In order to test the greater than "worst practical case" and determine a microbioaerosol dose for L. pomona MLS and S. typhimurium for hamsters and poults, respectively, aerosol experiments were conducted in an isolation chamber. These studies allowed the opportunity to test the Lundgren Electrostatic Aerosol Precipitator (LEAP) and the AGI aerosol samplers. Poults became infected when the ambient air contained about 5-20 x 10^4 S. typhimurium/liter of air (Tables 15 and 16). Hamsters did not become infected when exposed to similar aerosol concentrations of L. pomona MLS (Table 2).

Minimum infectious dose (MID) studies of both L. pomona MLS and S. typhimurium provided a tool for quantitation of infectivity/or infection potential or alteration thereof. Stock cultures of L pomona MLS had a MID of 20 cells when injected intraperitoneally (I/P) into male weanling hamsters (Table 17). The S. typhimurium MID was below 200 cells injected I/P for week-old poults (Table 4). Whenever a new series of experiments was conducted, the virulence was tested or had recently been determined. This was particularly important with the L. pomona MLS since quantitation was more precise for stored cultures and virulence was a more labile characteristic than with the S. typhimurium.

L. pomona MLS (serotype confirmed by the Center for Disease Control by cross agglutination) isolated from the MOD-A slurry 18 days post-

Table 13. HAMSTER EXPOSURE TO MICROBIOAEROSOL OF LEPTOSPIRA SEROTYPE POMONA MLS IN AN ISOLATION CHAMBER (five-minute exposure)

Inoculum Organisms/ml	Amount Nebulized (ml)	Agar Plates	LEAP	Results Positive/Possible	Organisms/ liter of air
Control-PBS	1.8	0/5	-	0/5	None
Undiluted culture ($< 2 \times 10^8$)	1.2	0/5	-	0/10	$< 6.9 \times 10^5$
2×10^8	1.1	0/5	-	0/10	6.9×10^5
2×10^7	1.1	0/5	-	0/9	6.9×10^4
2×10^5	1.8	0/5	-	0/8	1.1×10^3
2×10^3	1.8	0/5	-	0/7	1.1×10^1

Table 14. INFECTION OF TURKEY POULTS BY VARIOUS ROUTES OF INOCULATION (week-old turkey poults, 0.1 cc inoculum used)

Group	No. of *S. typhimurium*	Positive/Possible Ocular	Oral	Nasal
1	9.8×10^{-2}	0/3	0/3	0/3
2	9.8×10^{0}	0/3	1/3	0/3
3	9.8×10^{4}	0/3	1/3	0/3
4	9.8×10^{8}	1/3	3/3	2/3
5	9.8×10^{12}	0/3	2/3	0/3
6	9.8×10^{16}	3/3	3/3	3/3
7	Control	0/3	0/3	0/3

Table 15. TURKEY POULT EXPOSURE TO MICROBIOAEROSOL OF *SALMONELLA TYPHIMURIUM* IN AN ISOLATION CHAMBER

Inoculum (per ml)	Amount Nebulized (ml)	Results
9.8×10^{16}	3.0	9.3×10^{14} Sal/l of air 10/11 Poults
8.0×10^{6}	3.2	8.1×10^{4} Sal/l of air 1/9 Poults
13.0×10^{7}	3.6	14.9×10^{5} Sal/l of air 7/11 Poults
15.2×10^{6}	3.7	17.9×10^{4} Sal/l of air 0/12 Poults
18.7×10^{9}	3.4	20.0×10^{7} Sal/l of air 12/12 Poults

Table 16. SALMONELLA AEROSOL EXPERIMENTS CONDUCTED IN AN ISOLATION CHAMBER

Test	Results (Sal/l)	Procedures (or Observations)
Exp. 1 (A-1)	- - -	No poults exposed, experiment conducted to test production of and sampling of a salmonella aerosol. LEAP No. (5) and fall plate (3) (open Petri dishes) samples were negative for salmonella. Nebulizer not functioning.
Exp. 2 (A-2)	- - -	No poults. Used a Devilbis 640 Nebulizer. Results: (4) LEAP and (4) fall plates all positive for salmonella but TNTC (too numerous to count). Therefore, Nebulizer functioning and aerosol sampled.
Exp. 3 (A-3)	- - -	Eleven poults plus 5 fall plates exposed to a Salmonella typhimurium aerosol 3.0 ml of broth culture (9.8 x 10^{16} S. typhimurium/ml). Five 1-minute LEAP samples with 0.1 ml collecting fluid plated.
LEAP (Lundgren Electrostatic Aerosol Precipitator)	9.3 x 10^{14}	5 LEAP plates - TNTC* 5 LEAP plates - TNTC Poult tissue culture (positive/possible) Liver (4/11), Caecum (10/11), Cloaca (0/11), Oral (9/11)
Exp. 4 (A-4)	8.1 x 10^{4}	Nebulized 3.2 ml of 8 x 10^{6} S. typhimurium ml broth culture. Exposed 9 poults and 5 fall plates, and took five 2-minute LEAP samples No. of colonies/plate LEAP 1, 0, 1, 1, and 2 Fall plates 2, 0, 0, 83 and 2 Poults - 1 oral sample positive

Table 16. (continued) SALMONELLA AEROSOL EXPERIMENTS CONDUCTED IN AN ISOLATION CHAMBER

Test	Results (Sal/1)	Procedures (or Observations)
Exp. 5 (A-5)	14.9×10^5	Nebulized 3.6 ml of a 130×10^6 *S. typhimurium* 1-ml broth culture. Exposed 11 poults 5 fall plates and took five 2-minute LEAP samples. No. of colonies/plate LEAP 1, 4, 4, 1, 0 Fall Plates 0, 0, 0, 0, 0 Poults - 7 positive; liver (4/11) Caecum (4/11), oral (6/11; best)
Exp. 6 (A-6)	17.9×10^4	Nebulized 3.7 ml of a 152×10^5 *S. typhimurium*/ml broth culture. Poults were exposed and five 2-minute samples were taken. No. of colonies/plate LEAP 0, 0, 0, 0, 0 Fall Plates N.D. Poults (12) - All negative (caecum, cloaca, liver and oral).
Exp. 7 (A-7)	2.0×10^8	Nebulized 3.4 ml of a 187×10^8 *S. typhimurium*/ml broth culture. 12 poults were exposed and five 2-minute LEAP samples were taken. Three control birds not exposed. No. of colonies/plate LEAP TNTC Poults - 12 positive; Caecum (10/12), Oral (10/12), Liver (8/12)

*TNTC - Too numerous to count.

Table 17. INITIAL MINIMUM INFECTIOUS DOSE - QUANTITATION OF LEPTOSPIRA POMONA MLS VIRULENCE

Number of Leptospires inoculated	Kidney culture results (Positive/ No. inoculated)	Serum agglutination results (Positive/ No. inoculated)
0.2	0/5	0/5
2.0	0/5	0/5
20	1/5	1/5
200	1/5	3/5
2,000	3/5	3/5
20,000	1/5	3/5
200,000	2/5	5/5
2.0×10^6	5/5	5/5
20.0×10^6	2/5	3/5
20.0×10^7	3/5	5/5
0	0/5	0/5
0	0/5	0/5

seeding had lost all virulence according to MID studies with the organism (Tables 17, 18, 19, and 20). The significance of this finding cannot be understated. Does the virulence return with time or is it environmentally switched off and on? Survival in wastes may perpetuate in masked form a microbiological health hazard. A recheck was made of the MID of the stock Leptospira pomona MLS culture (Table 21). Saprophytic members of the genus Leptospira are commonly found in surface waters throughout the world. Environmental alteration of pathogenic leptospires may make them well-suited for continued existence in soils or waters should they survive waste disposal treatment conditions.

Table 18. MINIMAL INFECTIOUS DOSE STUDIES OF MARCH 24, 1972, LEPTOSPIRAL DITCH ISOLATES (studies in hamsters)

No. of Leptospires	Serum Antibody Titer (Positive/no. inoculated)	Kidney Culture
Control	0/5	-
2×10^0	0/5	-
2×10^1	0/5	-
2×10^2	0/5	-
2×10^3	0/4	-
2×10^4	0/4	-
2×10^5	0/5	-
2×10^6	0/5	-
2×10^7	0/5	-
2×10^8	0/5	-

Table 19. MINIMUM INFECTIOUS DOSE STUDY OF *LEPTOSPIRA* SEROTYPE *POMONA* MLS ISOLATED FFOM THE MANURE SLURRY OF MODEL OXIDATION DITCH A, ON MARCH 31, 1972 INOCULATED I/P INTO HAMSTERS

Group Number	Concentration (lepto/ml)	Culture of Kidney (positive/possible)	Serum Agglutination (positive/possible)
1	0 (control)	0/5	0/5
2	2×10^0	0/5	0/5
3	2×10^1	0/5	0/5
4	2×10^2	0/5	0/5
5	2×10^3	0/5	0/5
6	2×10^4	0/5	0/5
7	2×10^5	0/5	0/5
8	2×10^6	0/5	0/5
9	2×10^7	0/5	0/5
10	2×10^8	0/3	0/2

Table 20. MINIMUM INFECTIOUS DOSE STUDY OF LEPTOSPIRA SEROTYPE POMONA MLS STOCK CULTURE[a] INOCULATED I/P INTO HAMSTERS[b]

Group Number	Concentration (lepto/ml)	Culture of Kidney (positive/possible)	Serum Agglutination (positive/possible)
1	0 (control)	0/4	0/5
2	2×10^0	2/4	2/4
3	2×10^1	4/5	2/5[c]
4	2×10^2	5/5	4/5[c]
5	2×10^3	1/5	4/5
6	2×10^4	0/5	2/3
7	2×10^5	0/5	4/5
8	2×10^6	0/5	5/5
9	2×10^7	0/5	5/5
10	2×10^8	0/3	3/3

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[a] The stock *L. pomona* MLS had been stored at toom temperature in closed cabinets. These stock cultures were not subcultured since receipt in January, 1972.

[b] Second MLS study.

[c] Note failure of infected hamsters to seroconvert.

Table 21. MINIMUM INFECTIOUS DOSE OF STOCK LEPTOSPIRA POMONA MLS

Group Number	Concentration (lepto/ml)	Culture of Kidney (positive/possible)	Serum Agglutination (positive/possible)
1	(control)	0/5	0/4
2	2×10^0	3/5	2/5
3	2×10^1	5/5	1/1
4	2×10^2	4/5	2/2
5	2×10^3	4/5	1/1
6	2×10^4	5/5	0/2
7	2×10^5	5/5	0/5
8	2×10^6	4/5	1/3
9	2×10^7	5/5	2/4
10	2×10^8	4/5	2/3

Third MID study, March 21, 1973.

Table 22 summarized the Model Oxidation Ditch A sampling. The Rosemount sampling was simulated as shown by comparison of the bioaerosol counts. Again, the presence or absence of animals produces the largest difference in counts. The ditch was seeded with Salmonella per ml of ditch liquid. As indicated in Table 22, only minute quantities of air contain the indicator Samonella, and when animals were absent, no Salmonella were detected. A surprisingly large number of fecal streptococci were recovered compared to the Rosemount sampling. The reason is unknown. The difference is not due to the difference in sampler. Table 23 shows the results of a comparative study using the LEAP and the AGI. The similarity should be noted. Table 24 indicates greater microbioaerosol activity when hamsters were present over the laboratory model ditch. Note leptospires were isolated on all five samplings.

The University of Minnesota Rosemount Experiment Station field oxidation ditch (FOD) manure slurry was discovered to be culturally positive with an unidentified Leptospira in October, 1972. This was a consequence of culturally monitoring this manure slurry-water mixture for leptospires prior to seeding with L. pomona MLS in the MOD-A for experiment L.4.5 (Tables 25 and 25a). The presence of these "unknown" leptospires was not determined, however, until after the pathogenic L. pomona MLS had been inoculated into the manure. The long incubation time required for culture development prior to examination by darkfield microscopy was longer than the control period of one week. The isolate may have been a "saprophytic" leptospira since it did not agglutinate screening to six common "pathogen" antisera (serotypes hardjo, autumnalis, pomona, canicola, icterohemorrhagiae, grippotyphosa). A leptospiral antigen and hyperimmune serum produced in rabbits is being evaluated by the Center for Disease Control, Atlanta, Georgia, to confirm this possibility, or that this is a less common pathogenic leptospira serotype.

The discovery of a leptospira in manure and/or water from the field oxidation ditch (FOD) or well was interesting. Questions arose: What was the source of the leptospira? Are there carriers/cases in the herd? Can leptospires be detected in a field situation using the technique and aerosol sampling technique?

In order to answer these questions to some extent, the aerosol samples from the Rosemount studies (see Section V), the manure slurry of the MOD-A and the FOD, and the aerosol samples from the MOD-A (Table 23) were cultured. The presence of the leptospiral contaminant probably did not affect the experiment other than in a positive manner. For example, naturally occurring levels of leptospires can be detected and isolated by the filter-agar plate procedure. Previously, studies with this technique were with laboratory-adapted

Table 22. MODEL OXIDATION DITCH A - SAMPLING TURKEY POULTS AEROSOL DATA TAKEN WHEN TURKEYS PRESENT AND ABSENT

Viable Bacterial Aerosols (LEAP counts) on PCA (total, L-EMB (coliform), M-Ent (Fecal Streptococcus), and BGS enrichment (*Salmonella*), 37 C, 24 h4. Counts Given in Colony Forming Units/Liter (CFU/1)

	Poults Present				Poults Absent			
Organism	No. of Samples[a]	Total CFU/1	Range CFU/1	Average CFU/1	No. of Samples[a]	Total CFU/1	Range CFU/1	Average CFU/1
Total	35	585	0.7-173	17.00	12	57.4	0.40	1.4
Coliform	32	2.0	.02-0.6	0.90	10	00.02	0.00	0.2
F. Strep.	35	142	.02-26	04.00	11	00.10	0.2-.1	0.009
Salmon.	40	.009	.001-.003	4×10^{-5}	13	00.00	0.00	2×10^{-3}

(9 Salmonella were detected in 1.3×10^5 liters of air samples.)

(No Salmonella were detected in 4.3×10^4 liters of air sampled.)

[a] Each sampling represents 10,000 liters of air samples into approximately 25 ml of collecting fluid.

Table 23. COMPARISON OF COLONY-FORMING UNITS (CFU) PER LITER OF AIR SAMPLED WITH LEAP[a] ALL GLASS IMPINGERS (AGI) - MODEL OXIDATION DITCH-A (MOD-A) FIELD OXIDATION DITCH (FOD) AT SUMMER CONDITIONS

MOD-A Same Temp.				FOD[b]			
Date	EMB[1]	M-Ent[2]	PCA[3]	Date	EMB[1]	M-Ent[2]	PCA[3]
Oct 30, 1972	0	23	146	June 28, 1972	0	0	149
Nov 1, 1972	0	ND	6	July 5, 1972	0	0	37
Nov 7, 1972	0	17	503	July 18, 1972	0	0	82
Nov 12, 1972	0	6	33	Aug 9, 1972	0	0	6
Dec 21, 1972 (LEAP)	0	ND	40	Aug 8, 1972	1	1	176
Dec 28, 1972 (LEAP)	0	21	39	Sept 20, 1972	3	19	109
Total	0	67	767		4	20	559
X	0	11	128		1	4	96

[a] LEAP = Lundgren Electrostatic Aerosol Precipitator

[b] Selection of field data when external air temperature was between 15° C-30° C. Data is East Barn Data. (intramural)

1=Eosin Methylene Blue Agar; 2=M-Enterococcus Agar; 3=Plate Count Agar

Table 24. RESULTS OF AGI (ALL GLASS IMPINGER) SAMPLING OF MODEL OXIDATION DITCH A - ANIMAL HOUSING UNIT MICROBIOAEROSOL: ROTOR VELOCITY VARIED NOVEMBER 16-17, 1972 (Enumeration as Colony-forming Units/liter or CFU/l)

	Rotor Speed	PCA Aliquot No. 1	PCA[a] Aliquot No. 2	$\bar{X}$	Leptospires
Hamsters Present (Nov. 16, 1972)					
	Normal	102,116	97,130	111	Yes
	Fast	17, 13	2, 4	9	Yes
	Fastest	23, 36	17,29	26	Yes
Hamsters Absent (Nov. 17, 1972)					
	Normal	1, N.D.	1, 1	1	Yes
	Fast	2, 6	5, 5	4	Yes
	Fastest	N.D.	(splashing problem)	N.D.	N.D.

[a] PCA = Plate Count Agar/Tryptone Glucose Yeast Extract Agar

Table 25. ROSEMOUNT FIELD OXIDATION DITCH: MICROAEROSOL (AGI) AND SLURRY SAMPLING FOR LEPTOSPIRES

Sample	Date	Result (positive/possible)
Air	12/20/72	Negative, (0/22)
	12/27/72	Negative, (0/24)
	1/ 2/73	Negative, (0/24)
	1/10/73	Negative, (0/21)
Slurry	12/20/72	Negative, (0/3)
	2/20/73	Negative, (0/3)
	3/26/73	Negative, (1/3)

These samples were aliquots obtained from the AGI's used for routine microbioaerosol studies on these dates.

Positive result recognized in May, 1973.

Table 25a. MODEL OXIDATION DITCH: ANIMAL HOUSING UNIT MICROAEROSOL (LEAP) AND SLURRY SAMPLING FOR LEPTOSPIRES

Sample	Date	Result (positive/possible)
Air	12/18/72	Negative, (0/2)
	12/20/72	Negative, (0/2)
	12/22/72	Negative, (0/2)
	12/28/72	Negative, (0/2)
	12/29/72	Negative, (0/2)
	1/ 3/73	Negative, (0/2)
	1/ 4/73	Negative, (0/2)
	1/ 9/73	Negative, (0/2)
	1/11/73	Negative, (0/2)
	1/12/73	Negative, (0/2)
MOD-Slurry	12/18/72	Negative, (0/2)
	12/20/72	Negative, (0/4)
	12/27/72	Negative, (0/2) plus 1 (?)
	12/29/72	Negative, (0/3) plus 1 (?)
	1/ 3/72	Negative, (0/4) plus 1 (?)
	1/ 4/72	Negative, (0/6)
	1/ 8/72	Negative, (0/5)
	1/10/72	Negative, (0/5)
	1/12/72	Negative, (0/5)

pathogenic leptospires. Laboratory experience suggests that the oxidation ditch may be a "cultural vat" for leptospires. The isolation of leptospires from the FOD tends to confirm this contention.

Beef cattle associated with the FOD confinement feed operation were also studied. On March 1, 1973, blood samples were obtained prior to slaughter from 24 of the 36 beef cattle (Herefords) confinement-housed above the FOD at Rosemount. The results were interesting. All 24 sera were uniformly serologically negative by microscopic agglutination test against *Leptospira* serotypes *pomona*, *hardjo*, *grippotyphosa*, *canicola*, *icterohemorrhagiae*, and *autumnalis*, but positive at a 1:10 serum dilution against the "unknown" leptospiral ditch isolate. A reaction at the 1:10 dilution is considered non-significant, but the consistency of the agglutination for all 24 sera suggests possible significance in view of the fact that other routine sera tested from other state cattle herds submitted by veterinarians for agglutination screening were completely negative to this "unknown" leptospiral antigen.

Thirty-three of the 36 incoming replacement calves (Herefords) were bled for serum one week later (3/8/73). The information concerning the origin of these calves was not obtained, but they were kept at another area of the Rosemount Experiment Station for about three weeks prior to this time to be started on concentrate, feed, and roughage. Their sero-agglutination reaction pattern may indicate only vaccination or retained maternal antibodies, but *also* some reactions to the leptospiral "unknown" at the 1:20 dilution. These calves were bled again just prior to shipment for slaughter in August, 1973.

Animal models exposed to the model oxidation ditch-confinement housing unit were not overwhelmed with *L. pomona* MLS or *S. typhimurium* infection, nor did aerosol sampling of the ambient air with the LEAP indicate high levels of pathogen contamination of the air (Tables 7, 8, 25, 25a, 26, and 27). Hamsters confined over an *L. pomona* MLS-contaminated slurry did not show evidence of leptospirosis. Poults in 2 of 4 *S. typhimurium* experiments remained negative for signs of salmonellosis but in S.2.W., 20 of 148 and in S.3.5., 12 of 102 poults became infected with *S. typhimurium*. The overall low response rate may have been due to local tissue immunity (with lack of systemic immune involvement if the pathogens were present and contacted). It was evident further that the MOD-A successfully simulated microbioaerosol conditions present in a field oxidation ditch-cattle confinement unit (Table 23). The microbioaerosol contamination levels in oxidation ditch housing are less than those associated with traditional dairy farm operations tested.

Table 26. **ANIMAL EXPOSURE, MANURE SLURRY, AND AEROSOL SAMPLING RESULTS OF LEPTOSPIRAL/HAMSTER STUDIES UTILIZING MODEL OXIDATION DITCH-A**

Experiment	Control	1	2	3	4	5	6	7	8
Slurry L.1.W.	-	+	+	+	+	+	+	+	+
O. Ditch	0/10	0/10	0/5	0/5	0/4	0/5	0/5	0/10	0/14
Aerosol	-	-	-	-	-	-	-	-	-
Slurry L.2.W.	-	+	+	+	+	+	+	ND	ND
O. Ditch	0/5	0/5	0/5	0/5	0/5	0/5	0/5	ND	ND
Aerosol	-	-	-	-	-	-	-	ND	ND
Slurry L.3.S.	-	+	+	+	+	+	+	+	ND
O. Ditch	ND	0/5	0/5	0/5	0/5	0/5	0/5	0/5	ND
Aerosol	ND	-	-	-	-	-	-	-	ND
Unknown Slurry L.4.S.	+	+	+	+	+	+	+	ND	ND
O. Ditch	0/5	0/5	0/5	0/5	0/5	0/5	0/19	ND	ND
Aerosol	-	-	-	-	-	-	+	ND	ND

Table 27. LEPTOSPIRAL ANIMAL FEEDING AND RECYCLED FEED SAMPLE RESULTS

Experiment	Control	1	2	3	4	5	6	7	8
Slurry L.1.W.	-	+	+	+	+	+	+	+	+
Fed	0/5	ND	0/5	0/5	0/5	ND	ND	0/5	0/5
RCF	-	-	-	+	+	-	-	+	+
Slurry L.2.W.	-	+	+	+	+	+	ND	ND	ND
Fed	-	0/5	0/5	0/5	0/5	ND	ND	ND	ND
RCF	ND	-	-	-	+	ND	ND	ND	ND
Slurry L.3.S.	-	+	+	+	+	+	+	+	ND
Fed	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	ND
RCF	-	-	+	-	+	+	+	ND	ND
Slurry L.4.S.	+	+	+	+	+	+	+	+	+
Fed	ND	ND	0/5	0/5	0/5	0/5	ND	ND	ND
RCF	-	-	-	+	+	+	-	ND	ND

It is interesting to note that on the one date that leptospires were detected in the MOD-A housing unit, testing was conducted for microbioaerosol production at various rotor speeds. The "normal" rotor speed was that derived from empirical experience as producing the greatest rate of slurry flow. It apparently also produces the greatest respiratory hazard. The other speeds are not practicable.

SUMMARY

Evaluation of laboratory data collected from research grant #R802205 suggests that the oxidation ditch aeration by rotor is probably not a public health hazard in terms of aerosol dissemination of L. pomona MLS and S. typhimurium. However, if the contaminated manure slurry is not disinfected in some manner, environmental health problems would probably result. Survival studies have demonstrated that pathogenic leptospires (L. pomona) persist for 183 days in sterile soil supersaturated with water and kept at 20° C (18); however, the authors did not conduct virulence studies. Many cases of leptospirosis associated with contaminated streams and recreational waters are recorded. These cases, coupled with previous work describing leptospiral viability for 138 days in the cattle manure slurry of a model (Pasveer) oxidation ditch, suggest this problem is real. Leptospires isolated from the manure slurry of the Model-A oxidation ditch were serologically identical to those L. pomona MLS inoculated into the slurry. The oxidation ditch may serve as a "culture vat" and reservoir for leptospires because detection of viable leptospires for prolonged periods requires multiplication of the microorganisms. Previously, the finding of sufficiently high levels of Thiamine and Vitamin B_{12} in the manure slurry suggested this possibility since a source of nitrogen and oxygen is also provided.

Although the ditch isolates remained stable antigenically (as L. pomona MLS), the virulence (MID) studies demonstrate a decreased virulence of the organism. The fact that virulence alters with environment is not surprising, but is significant to public health/environmental health concern because the existence of a potential pathogen in the uncontrolled environment could make it possible for the attenuated pathogen to enter through many of the myriad routes of disease transmission into another susceptible host, after which the attenuated organism could revert to its original state, thereby causing disease. In fact, survival in a non-host associated environmental cycle is a new line of leptospiral disease transmission not seriously considered previously. As improved methods of detection become available, the role of leptospires and salmonella in public health will become more apparent. Failure of these organisms to cause seroconversion after infection hinders these efforts, but again, opens possible routes not before considered because of the "masking" of infection not detectable with present diagnostic methods used.

Our findings of an "unknown" pathogenic saprophytic leptospiral serotype in the Rosemount manure slurry and seroconversion of exposed cattle serve to demonstrate exposure potential. The cattle either brought the leptospires to the ditch or became infected from it.

In summary, it might be well to state that the oxidation ditch as a means to an end (waste treatment) also serves as an instrument in creating channels of disease transmission, but is not a cause. It can serve as a dead end if a loss of virulence occurs or if a method of disinfection is provided. Prevention of disease in animals so housed is essential to the protection of the inhabitants of an environment (both man and animal) from disease, whatever that disease might be.

SECTION V

STUDY OF THE BIOAEROSOL PRODUCTION OF A FIELD OXIDATION DITCH

INTRODUCTION

Purpose

The purpose of this study was to monitor the bioaerosol production from a rotor-aerated, operational field oxidation ditch (Pasveer) and to assess the public health hazard in relation to bacteria produced by this waste treatment facility.

Importance

The importance of this study can be determined by consideration of the following:

1. Langmuir (19) presents a historic review of the literature citing cases of airborne infection.
2. The droplet nuclei theory proposed by Wells (20) whereby a particle originating from a liquid becomes small enough to enter the lung.
3. The oxidation ditch is a treatment area with high concentrations of enteric bacteria, some of which may be pathogenic for man and/or animals.
4. Animals housed on slats over the ditch and people working in the area are exposed to airborne pathogens.

Description of the Oxidation Ditch Operation

The field oxidation ditch (Figure 5) is one of the facilities at the University of Minnesota's Experiment Station located at Rosemount, Minnesota. The oxidation ditch is located within the Livestock Research Building on the Agricultural Engineering Farmstead (Figures 6 and 7). Two enclosed housing units have been constructed over the oxidation channel. Within each unit, 18 beef cattle were fattened on ordinary grain and concentrate typical of usual confinement production units. Urinary and fecal wastes passing through the slotted floors of the housing units combined to become the manure slurry of the Pasveer aerobic treatment process. A rotor at the west end of the oxidation channel served to propel and aerate the waste material. The rotor and oxidation channel were covered by the housing unit and the

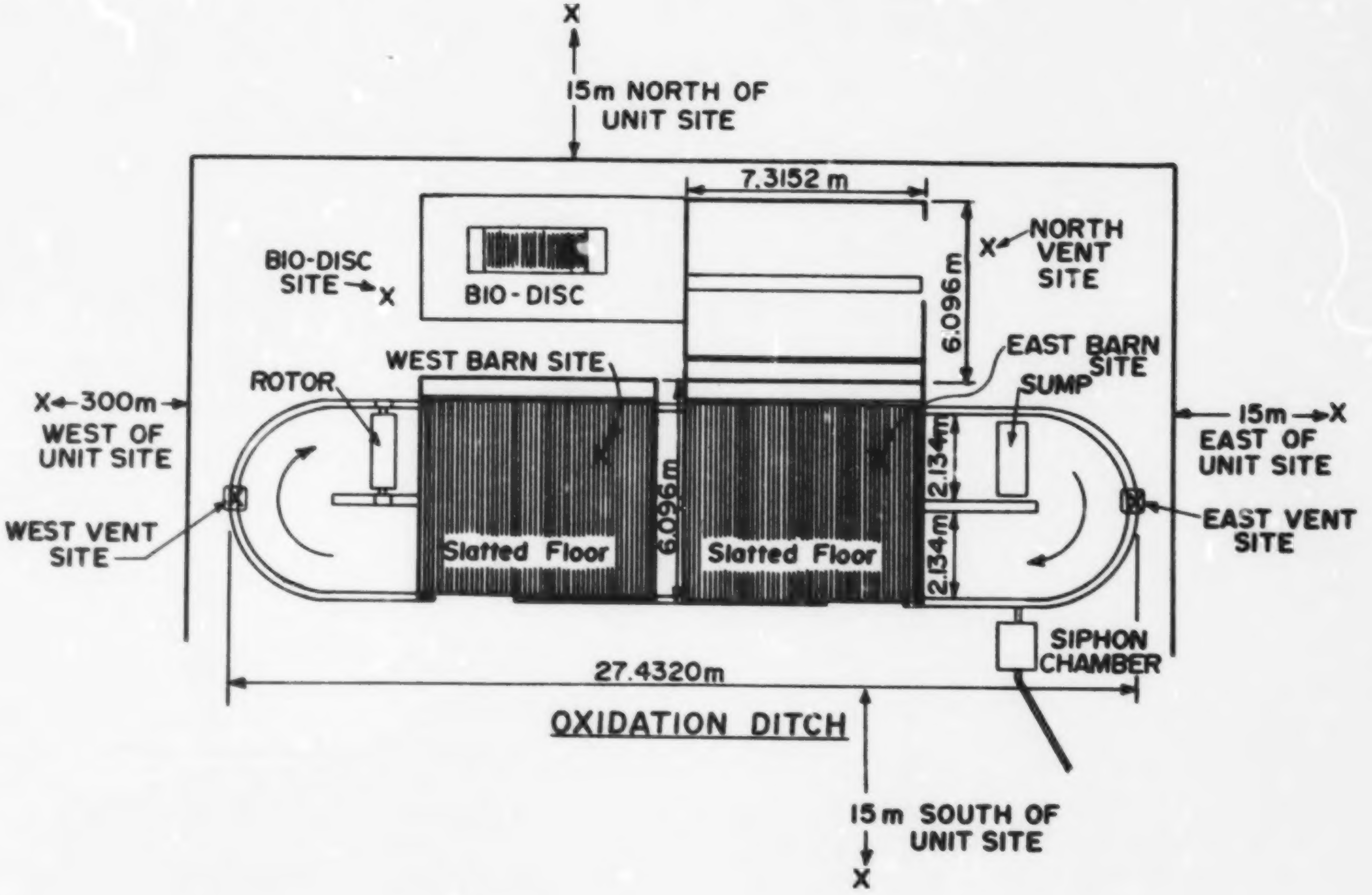
X
15m NORTH OF UNIT SITE
7.3152 m
X NORTH VENT SITE
BIO-DISC SITE X
BIO-DISC
6.096m
WEST BARN SITE
EAST BARN SITE
SUMP
ROTOR
X 300m WEST OF UNIT SITE
15m X EAST OF UNIT SITE
2.134m
2.134m
6.096m
WEST VENT SITE
EAST VENT SITE
Slatted Floor
Slatted Floor
SIPHON CHAMBER
27.4320m
OXIDATION DITCH
15m SOUTH OF UNIT SITE
X

Figure 5. Oxidation ditch at Rosemount

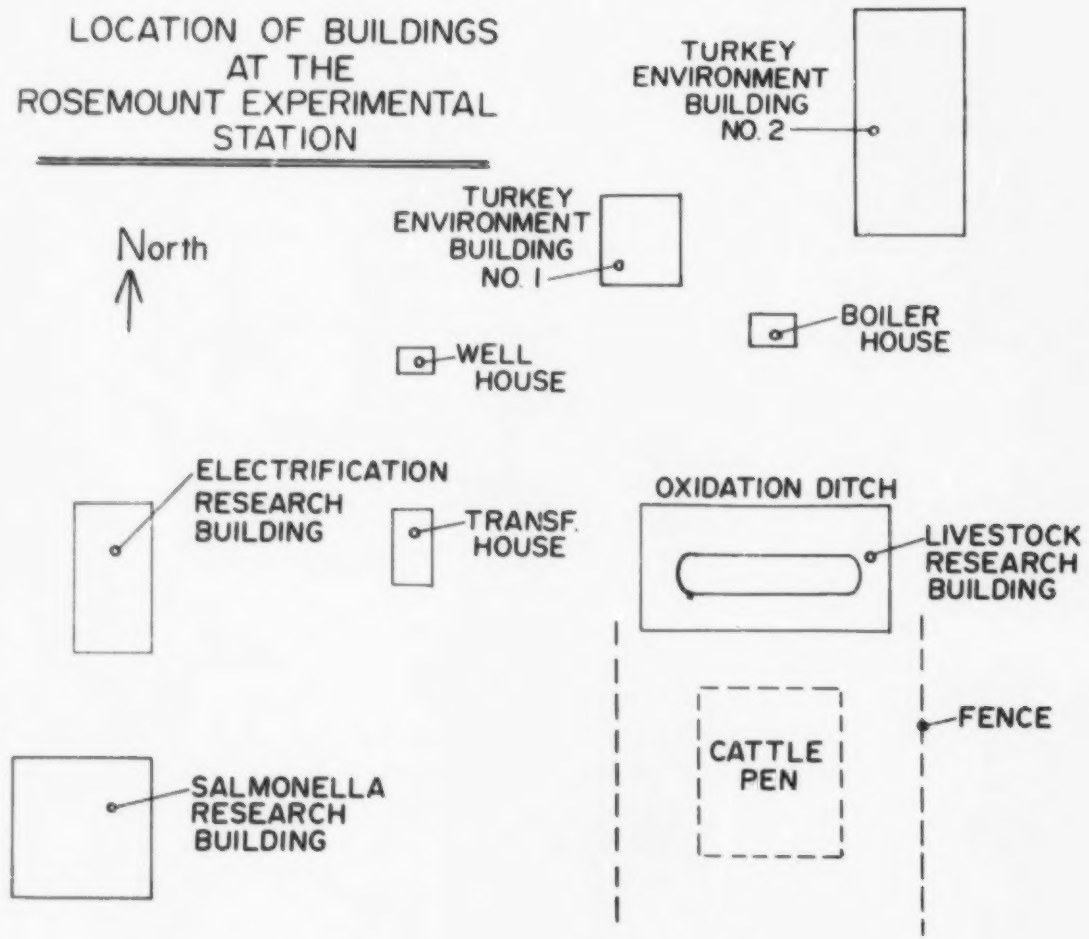
LOCATION OF BUILDINGS
AT THE
ROSEMOUNT EXPERIMENTAL
STATION
North
TURKEY ENVIRONMENT BUILDING NO. 2
TURKEY ENVIRONMENT BUILDING NO. 1
BOILER HOUSE
WELL HOUSE
ELECTRIFICATION RESEARCH BUILDING
TRANSF. HOUSE
OXIDATION DITCH
LIVESTOCK RESEARCH BUILDING
FENCE
CATTLE PEN
SALMONELLA RESEARCH BUILDING

Figure 6. Rosemount farmstead buildings

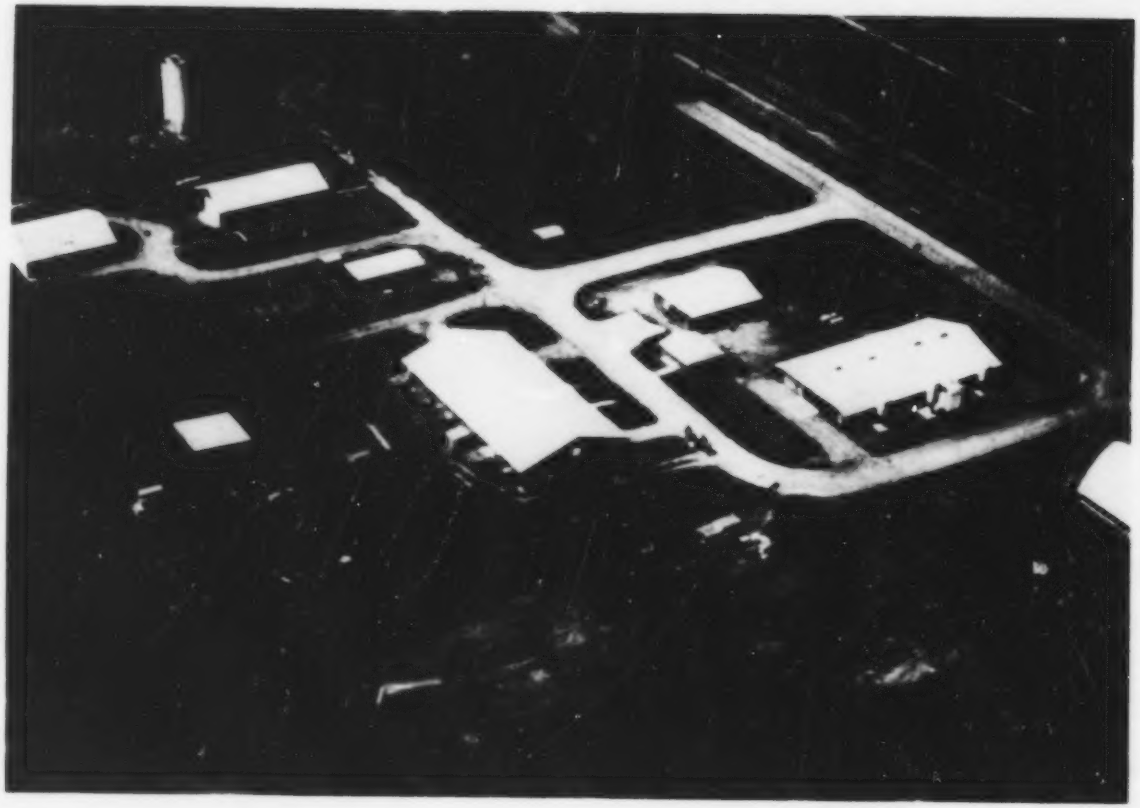

Figure 7. Rosemount farmstead aerial photo

rotor enclosure. Two vents, one at each end of the ditch, allowed air to exit from the ditch. Initially, vent fans drew the air from the ditch and animal units. During the preliminary studies, a change was made to positive pressure units which forced air continually into the units. The air was forced down through the slats and exhausted through the vents. The slat width space in the west unit was 3.81 cm and 3.18 cm in the east unit. There was a noticeable difference in exhaust air speed between the two units, the west vent passing more air than the east. This may have been due to the slat width. The normal operation of the oxidation ditch was interrupted occasionally by mechanical problems or experimental intervention. All such alterations are noted in sampling reports.

Making Two Time Segments of the Research Effort Was Important

The initial period of preliminary studies was utilized to develop experimental procedures and gather baseline information for development of standard protocol. A year-long study was then conducted with the developed protocol during which time the field and laboratory situations were stabilized.

MATERIALS AND METHODS

In order to monitor the air for bioaerosols, a program of sampling was developed.

Phase I, Preliminary Field Sampling Program

A preliminary program to develop field and laboratory procedures and gather data and reference material occupied the first 10 months of the 22-month study.

Field Work --

Sampler - Three different air samplers were evaluated: The All Glass Impinger (AGI) (Figure 8), the Lundgren Electrostatic Aerosol Precipitator (LEAP) (Figure 9), and the Casella Slit sampler. Although each of these devices has its unique advantages and commendable qualities, the AGI sampler was designated as the apparatus for routine monitoring in the field. The LEAP and the Casella were too cumbersome for use in the extramural and intramural environments in this study. Both the LEAP and Casella sampled a wide range of particle sizes. Interest was only in the 1 and 5 micron (μ) size particles which can penetrate to the depths of the pulmonary alveoli in the lung. The AGI was a better sampler for this purpose because it theoretically samples particles in the size range of 1-10 microns. A simultaneous sampling with

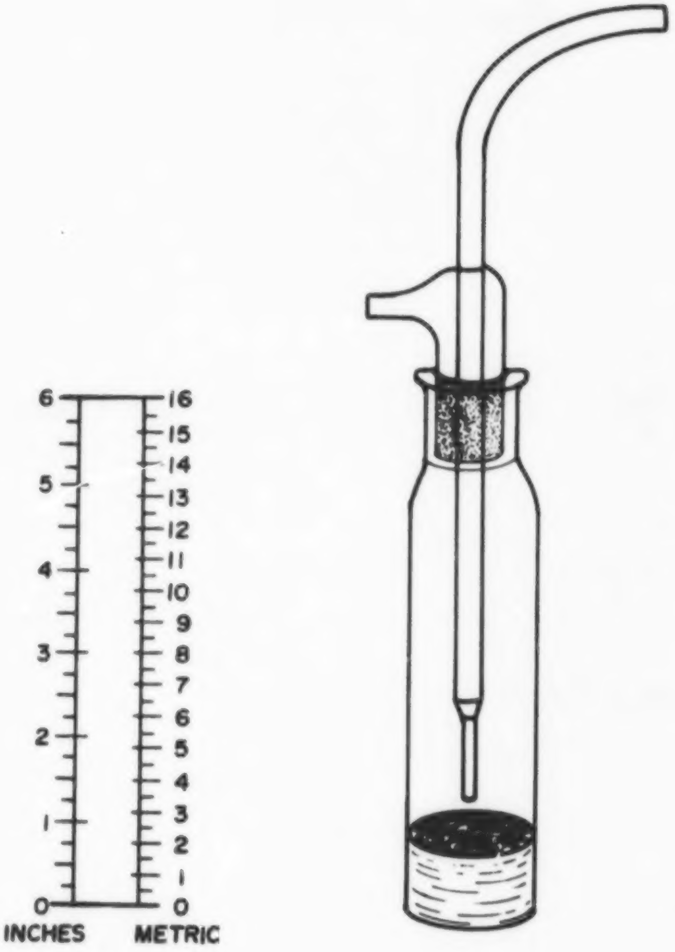
6
5
4
3
2
1
0
INCHES
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
METRIC

Figure 8. All glass impinger (AGI)

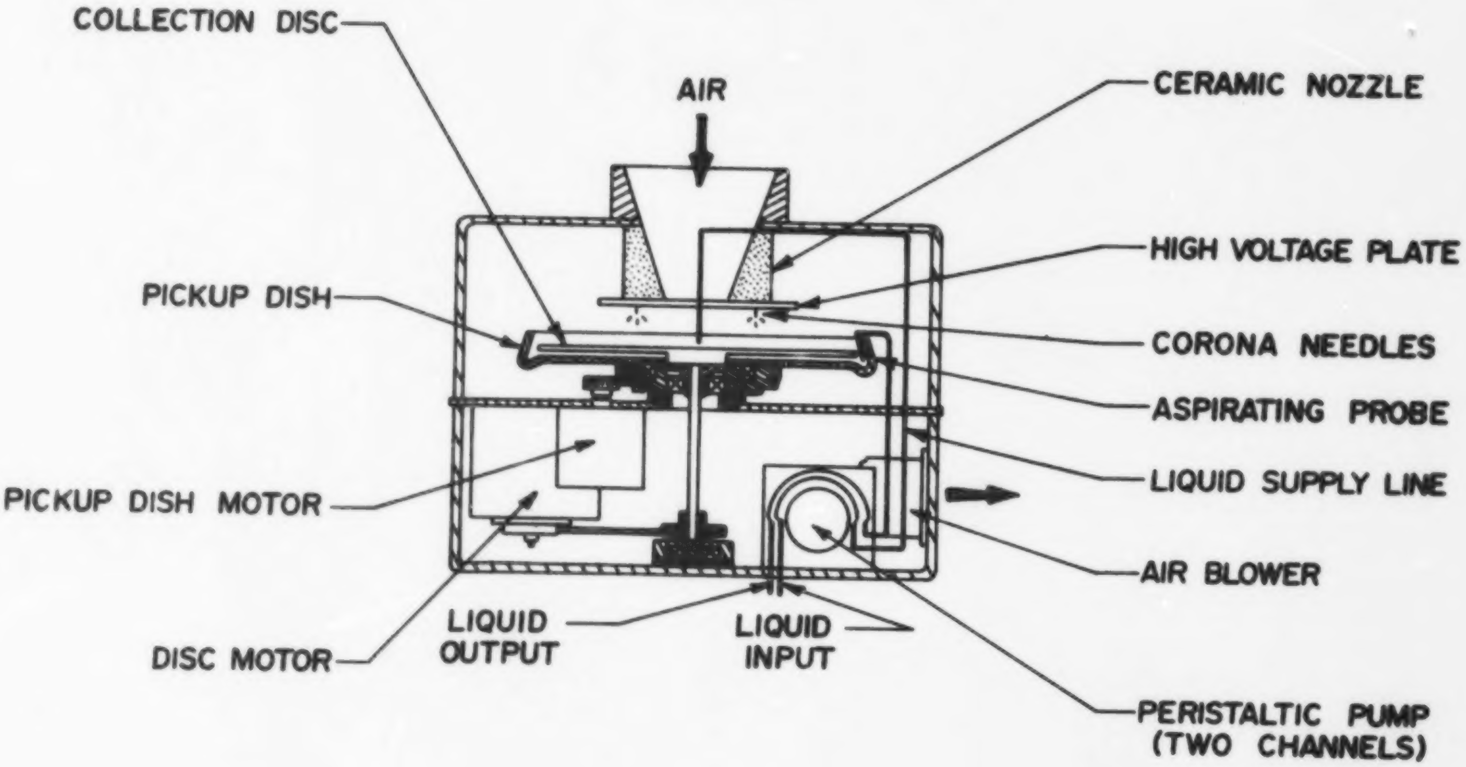
COLLECTION DISC
AIR
CERAMIC NOZZLE
HIGH VOLTAGE PLATE
PICKUP DISH
CORONA NEEDLES
ASPIRATING PROBE
PICKUP DISH MOTOR
LIQUID SUPPLY LINE
AIR BLOWER
LIQUID OUTPUT
LIQUID INPUT
DISC MOTOR
PERISTALTIC PUMP (TWO CHANNELS)

Figure 9. Lundgren electrostatic aerosol precipitator

the AGI and the Casella (Table 28) was conducted. The AGI yielded 20 times the number of colonies per liter of air sampled than did the Casella. This meant that approximately one particle contains 20 colonies, indicating that particles sampled by the AGI harbor one or more bacterial colony-forming units. A study of the efficiency of particle collection done by May (21) also found that the impinger produced highly variable results.

The AGI has several notable advantages. It is designed to simulate the human respiratory system both with respect to sampling rate (12.5 liters per minute) and particle size retention (1-10 microns). Consequently, it was ideal for a study of health hazards. It is relatively inexpensive and permits the simultaneous sampling of multiple sites with little extra effort as compared to the LEAP or Casella samplers.

Collecting fluid - A number of trials compared different sampling fluids to be used in the AGI apparatus. These data are summarized in Table 29. It is evident that collection fluids containing peptone yielded higher counts. Ultimately a 0.1% peptone and 0.01% antifoam fluid was adopted as the standard for use with the AGI protocol.

Determination of variables - The first six months of preliminary work were used to determine field and laboratory variables that could affect the aerosol analysis. Sources of variation in field data studied were as follows:

1. Variability of duplicate samples.
2. Hourly variation.
3. Noticeable disturbances.
4. Sample storage.
5. Duration of sampling.
6. Ditch bacterial concentration.
7. Laboratory techniques.

Variables that could be controlled were sought. This was difficult during this time due to continuous variation in the sampling procedure, but some insights were gained. As the sampling protocol became standardized, so did the variables.

Contributing aerosol counts due to outside factors unrelated to the ditch or animals need to be determined. The three-sided barn which enclosed the housing units and the oxidation ditch also contained two

Table 28. COMPARISON OF AGI AND CASELLA COUNTS OF COLONY-FORMING UNITS, DIFFERENT SAMPLING SITES (colony-forming units/liter)

Site	Casella	AGI Counts
West Vent	18/liter	51/liter
East Vent	10/liter	150/liter
West Barn	12/liter	225/liter
North Vent	12/liter	12/liter
Downwind	1/liter	4/liter
$\bar{x}$	11/liter	88/liter

Table 29. EFFECT OF AGI COLLECTION FLUID ON OBSERVED AEROSOL COUNTS

	Mean viable airborne count CFU/liter			
Location	0.85% NaCl	1.0% Peptone	1.0% Peptone & 0.1% Antifoam	0.1% Peptone & 0.1% Antifoam
West Vent	11	39	20	18
East Vent	--	--	17	16
West Barn	59	118	57	60
East Barn	--	--	68	89
Rotor	7	26	--	--
Field	0.5	0.5	--	--

other experimental units close by which possibly posed some influence on the tests being conducted. For example, an exhaust fan from a north barn (Figure 5) was vented into an area adjacent to the east vent of the ditch. Further, exhaust from the biodisc room (Figure 5) was vented into an area near the west ditch vent. Samples taken at these sites showed that there was no detectable intermingling of airborne bacteria. The samples were taken one meter in front of the vent, the 30-cm and 100-cm heights are lower. At a distance of another 600 cm, the counts were half those at 1 meter. Therefore, at the east vent which is 15 meters away, there probably was no interference. Likewise, interference from the biodisc at the west vent was not observed. It was concluded that there were no contributing bacteria from the north vent and biodisc being collected at the east and west vent of the oxidation ditch (Table 30).

Table 30. EVALUATION OF POSSIBLE CONTAMINATION SOURCES

	NORTH VENT		
	30-cm height	100-cm height	160-cm height
Number of samples	33	39	32
Average total CFU/l	7	5	19
	BIO DISC LOCATION		
	30-cm height	100-cm height	160-cm height
Number of samples	9	13	13
Average total CFU/l	9	7	10

Variability of duplicate samples - The preliminary samples of air showed that simultaneous duplicates differed by as much as 50%. An understanding of this variation led to an investigation of possible variables. A variation of air volume sampled was ruled out as a factor. The airflow measured through the AGI's (12.5 liters/minute) was the same in all cases when checked using three different vacuum pumps. To ensure that the duplicate AGI's were sampling a uniform aerosol, a cylinder was devised which limited the air mass sampled by two AGI's (Figure 10). The cylinder was 15 cm in diameter, 43 cm long, and

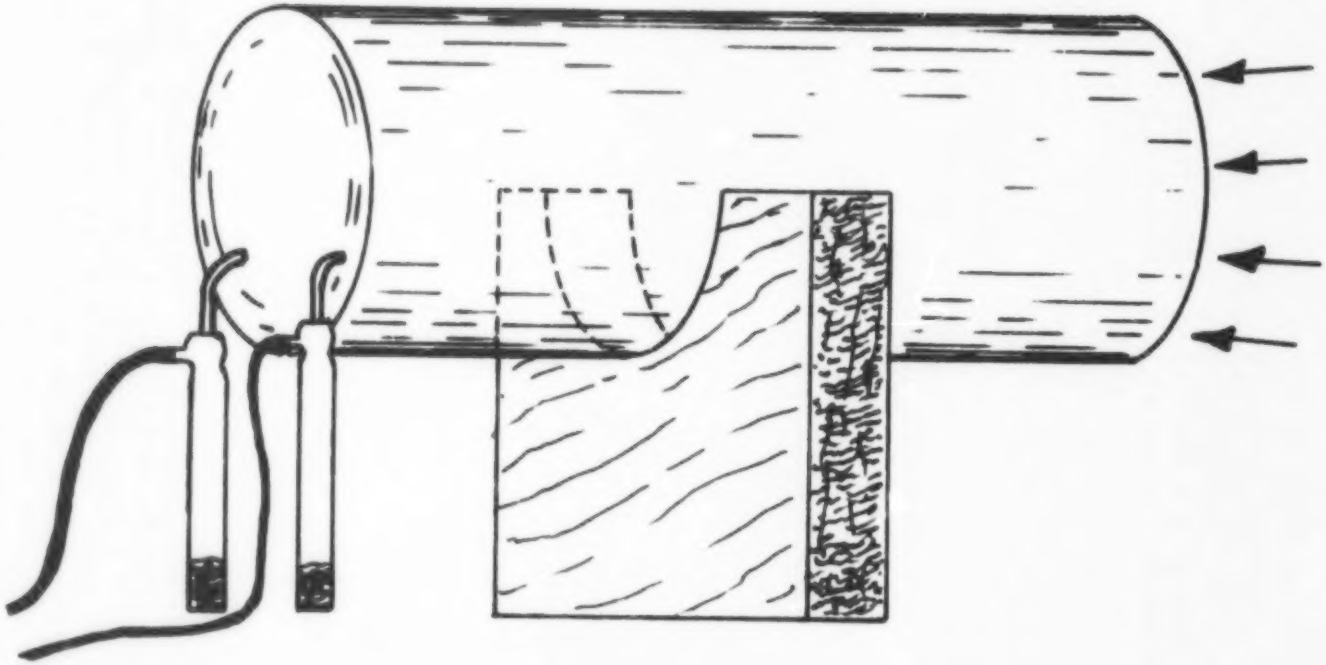

Figure 10. Cylinder for simultaneous AGI sampling

closed at one end except for two 1-cm openings. AGI nozzles sampling through the apertures produced variable counts, indicating that the bioairmass was not a homogenous mixture (Table 31). Higgins (22) and May (21) also found with an impinger that bioaerosol counts fluctuated a great deal. In Table 31 note that results from samplers 5 and 6, and from 7 and 8 vary widely. The reason for this was the occasional collection of large particles containing many bacteria. The impinging breaks up the agglomeration to produce multiple colonies when plated. In cases where duplicate results vary unrealistically, the result indicating contamination has been deleted. In some cases, even mosquito legs had been collected.

Table 31. SIMULTANEOUS DUPLICATE AIR SAMPLING WITH A CYLINDER
(colony-forming units/liter)

Location	Sampler	Total plate determination
Field	1	1
	2	3
North of unit	3	0.3
	4	0.7
South of unit	5	0.4
	6	80
West barn	7	56
	8	2659
	9	26
	10	43
East barn	11	37
	12	16
	13	86
	14	97
East vent	15	8
	16	4
East of unit	17	6
	18	3

Hourly variation - Early samplings showed that sites where the greatest variation in counts occurred were in the animal housing units. In order to understand whether or not variation was due to some factor which could be controlled, such as the operator's presence or movement, a series of hourly tests was taken in the west housing unit (Table 32 and 33). It was observed (afternoon, Table 32) that many of the cattle were lying down, as opposed to the morning when most of the cattle were standing. The higher afternoon counts might then have been contributed by incomplete venting of air due to the animals blocking the air that would be passed through the slats. But, another day of hourly sampling with careful notation of numbers of animals lying vs. standing did not substantiate the hypothesis. The hourly variations on Table 32 vary from 66 CFU/1 to 334 CFU/1 and those of Table 33 from 18 CFU/1 to 66 CFU/1. The change in relative humidity (R. H.) from hour to hour did not have any apparent influence on the counts as is also seen on Table 33. The cause of the fluctuations remained undetermined.

Noticeable disturbance - Often, drastic variations in counts could be predicted from certain activities in the experimental area. High bioaerosol counts were always observed when there were strong gusts of wind, people working in the surrounding area, or feed bins being filled. When such conditions existed, sampling was avoided if possible; however, when it was not possible, the results of the bioaerosol counts have greater than usual variations. Such a case occurred during sampling simultaneously at three heights at the vent exhaust when a gust of wind occurred. The results were: at 30 cm, 247 CFU/1; at 100 cm, 6 CFU/1; and at 160 cm, 1 CFU/1. When a noticeable disturbance as above occurred, the abnormal count was omitted (the 247).

Storage of samples - The storage time and temperature of the AGIs during collection and bacteriological plating, another step in the sampling procedure, was checked as a possible source for count variation since the collecting fluid could serve as a growth substrate. The time period between collection and plating was kept at 2 to 3 hours. The temperature was maintained at around 5° C by keeping the AGIs in an ice chest in the summer and in the winter in the chest with no ice located in a 10° C room. On a hot day, duplicate samples were compared by putting one of the duplicates in the cooler and the other at ambient temperature of 27° C. The bioaerosol counts showed no difference. Another check was done by comparing counts of samples taken from an AGI immediately and those kept two hours. The two counts were identical.

During preliminary sampling in the winter, the collection fluid froze after 0.5 minute of sampling. Later, sampling times were prolonged by addition of heat and storage of samplers in a heated area prior to use.

Table 32. AEROSOL FLUCTUATIONS ASSOCIATED WITH TIME IN THE WEST BARN (100 cm)

Time	Samples taken	Total CFU/l average	Range
9:30 a.m.	5	183	69-305
10:30	5	66	47-96
11:30	4	94	60-123
12:30 p.m.	3	334	240-515
1:30	3	251	223-269
2:20	2	241	208-274

Table 33. AEROSOL FLUCTUATIONS ASSOCIATED WITH TIME IN THE WEST BARN (100 cm)

Time	RH %	Temp. °C	Number laying (17 total)	Samples taken	Average total CFU/l
10:30 a.m.	87	16	3 laying	2	66
11:30	78	15	8 laying	2	53
12:30 p.m.	70	14	9 laying	2	18
1:30	70	14	4 laying	2	24
2:30	74	15	0 laying	2	45

Duration of sampling - The length of sampling time was an important aspect to observe when analyzing causes for variation since, hypothetically, some bacteria battered about in the agitated collecting fluid during sampling may not survive.

A field experiment compared AGI's sampling the air of the west barn for two times, 3 and 10 minutes. One set of AGIs sampled the air for 3 minutes concurrently and one, 10-minute AGI sample was begun. After the first three-minute sample ceased, a second set (of 2) three-minute samples was collected. The results are given in Table 34. The 10-minute sample was diluted 1:10 before plating. The results indicate that one 10-minute sample was an average of the two consecutive 3-minute samples; however, the other 10-minute sample was the same as the low counts. An additional test was done in the laboratory with collecting fluid from two AGIs that had collected air for five minutes. The total aerobic colonies per ml of fluid in the AGI was determined in the normal way (see below). The AGIs then sampled air from a clean area (set just inside a refrigerator with the door open 2 cm) for 10 additional minutes. The colonies per ml in both cases of the additional 10-minute sampling was nearly half of the original counts. These data implied that to compare counts from one area to another the sampling period should be the same. This knowledge was incorporated into our final sampling procedure. The air at every site associated with the field oxidation ditch was sampled for five minutes.

Table 34. EFFECTS OF SAMPLING FOR VARIOUS LENGTHS OF TIME
IN THE WEST BARN
(colony-forming units/liter)

Time of sampling	Average total
First set of 3 minute samples	271
Second set of 3 minute samples	94
Simultaneous 10 minute sample	187
First set of 3 minute samples	49
Second set of 3 minute samples	90
Simultaneous 10 minute sample	53

Ditch bacterial concentration - A potential variable which may have influenced the production of a bioaerosol from the ditch was the concentration of bacteria in the oxidation ditch. Higgins (22) and Darlow (23) found that with increasing bacterial concentrations of liquid from which droplet nuclei were formed the bioaerosol of such bacteria also increased. On five different days, during different seasons, when ditch samples were taken the bacterial counts varied little (Table 35). Total aerobic counts per ml of ditch liquid averaged 50 x 10^6 and ranged from only 15 x 10^6 to 92 x 10^6. Coliform organisms per ml of ditch liquid averaged 8.9 x 10^4 and ranged from 2.7 x 10^4 to 20 x 10^4. Fecal streptococci organisms per ml of ditch liquid averaged 15 x 10^4 and ranged from 2 x 10^4 to 32 x 10^4. The minor change in concentration is not believed to enter in the fluctuation of airborne counts.

Table 35. NUMBER OF BACTERIA IN ROSEMOUNT DITCH

Sample date	Total (CFU/ml)	Coliforms (CFU/ml)	F. Streptococci (CFU/ml)
Sept. 27, 1972	18,800,000	40,000	20,000
Oct. 3, 1972	15,000,000	140,000	170,000
Feb. 20, 1973	47,000,000	27,000	240,000
Feb. 28, 1973	77,000,000	36,000	20,000
March 28, 1973	92,000,000	200,000	320,000
Average	50,000,000	89,000	150,000

Approach to the study - The obvious critical parameters influencing the bioaerosol fluctuations during the sampling procedure have been evaluated above. There are still fluctuations that have not been explained since to discover them was beyond the scope of this project. The field study was used for evaluation of the health hazard of an operational Pasveer oxidation ditch. It was concluded that the whole cannot be subdivided into esoteric parts when dealing with problems, especially of environmental concern.

Laboratory - Once determined, standards of bacteriological culturing and laboratory protocol did not play an important role in the count variations. Laboratory personnel did not change, and all samples were treated the same once the standard protocol was established. Various methods or procedures were tested to determine the one most routinely reproducible, efficient, and inexpensive to suit our requirements. Each investigator of a new environment or new problem must develop his own laboratory procedures.

1. Dilutions. Each AGI was etched to mark a volume of 30 ml. The collecting fluid was added to this line. During sample collection some of the AGI fluid evaporated. In the laboratory, the collecting fluid in the AGI was made up to the 30-ml volume with sterile distilled water after sampling so that the proper dilutions could be made. The only alteration of this procedure was the dispensing method. An Erlenmeyer wash bottle apparatus was finally devised to dispense the liquid and was suitable for continuing use.

2. Plating. It was determined that plating of aliquots by either a "pour plate" or membrane filter technique provided satisfactory and comparable results. However, since both of these practices were time-consuming and cumbersome for the processing of many samples in a reasonable short peiod, a "surface spreading" technique which provided counts sufficiently accurate for this work was used.

   For total aerobic bioaerosol determination, Plate Count Agar (PCA) (Difco) was used as recommended in Standard Methods. Aliquots of AGI collecting fluid were surface spread on PCA. When dry, the plates were inverted, incubated 24 hours (48 hours after preliminary work) at 27° C, then counted, and total, colony-forming units per liter of air sampled was calculated. All plating was done in a hood.

3. Characterizing Bacteria. In an attempt to characterize the microbes in the aerosol, a variety of classical identification techniques were employed on "typical" colonies picked from enumeration plates (gram stain, hemolysis, catalase, Salmonella enrichment, etc.) In the preliminary experiments, Bacillus sp., Staphylococcus sp., and Corynebacterium sp. were the predominant types appearing in the aerosol. These organisms are commonly found in the air but were not necessarily associated with fecal excretion. Subsequent trials to detect enteric microorganisms involved direct plating of AGI fluid aliquots onto differential and selective media. The enteric indicator organisms chosen to be studied were

fecal streptococci (F. Strep.) and the coliform group (24, 25).

These organisms were chosen because there are well defined steps leading to their identification and they are found in the enteric tract. The two organisms behave quite differently owing to the differences in gram stain, cell wall, and motility. The differences offer possible explanations in ability to become airborne and to survive airborne exposure and detection.

It was found that coliform (gram negative, non-spore forming, lactose fermenting) detection was better made on Levine's Eosin Methylene Blue (L-EMB) agar. On Eosin Methyline Blue (EMB) (less specific) numerous non-coliform colonies grew, thus requiring further time and testing. Comparison of the ability to detect actual coliform organisms on L-EMB and EMB showed that the two media were essentially similar. To further test the suspected colonies picked from L-EMB (large and pigmented), a gram stain was done and the colony growth on Triple Sugar Iron (TSI) was checked for lactose fermentation.

An enrichment procedure is generally done on samples (Standard Methods). However, this was not feasible and the procedure would have differed from that of identifying total bioaerosol. A comparison was made, however, to see whether or not enrichment increased the coliforms. From 60 AGI samples the same aliquots were placed on L-EMB as in Brilliant Green Bile broth (BGB) enrichment. These were then streaked onto L-EMB. The results were the same, indicating that the direct plating without enrichment was adequate for our purposes.

The selective m-Enterococcus (m-ENT) agar was utilized for identification of F. Strep. Therefore, another selective agar, Bile Eschelin Agar (BES) (Difco) was used along with the catalase test.

Preliminary Field Data --

During the six months of the preliminary studies, thirty-two sample visits were made to the Rosemount site. More than 500 individual samples (totaling several thousand liters of air) were obtained at different locations inside and outside the animal housing units and oxidation ditch, at different heights above grade, and different distances from the ditch. This series of studies concerned itself with the problem of determining the quantity of bacteria present in the ambient air of an operational oxidation ditch.

The data obtained from these samples were analyzed to develop final laboratory and field protocol which would be the standard for a one-year study of the field oxidation ditch bioaerosol status and public health hazard. In other words, the critical sampling sites, the mean aerosol counts associated with these sites, and the magnitude of fluctuation that could be anticipated with time and location were identified. Characteristic data from the preliminary study are summarized in Tables 36 and 37. These data are from a procedure that was constantly changing; for example, samples were taken from various heights, sampling durations varied, different amounts of collecting fluid were cultured, different collecting solutions were used, and ditch conditions were dissimilar. The ventilation system in the housing units was altered. Until March, 1972, the housing units and ditch had a negative pressure airflow since exhaust fans were utilized. At that time, the exhaust fans were removed and intake fans placed in the housing unit to create a positive pressure flow in the units. The ditch was intermittently operational and the rotor cover was sometimes removed. The ditch cover over the east end of the ditch was occasionally removed (thus eliminating a point source for exhaust). Generally, 3- to 15-minute duration air samples were made with the AGI, and samples were taken at 30- to 100-cm heights. Duplicate samples were taken at each site. In the laboratory, 0.5 to 5 ml of collecting fluid was cultured by a pour plate method and incubated 24 hours at 27° C, colonies were counted, and calculations of "total colony-forming units per liter of air sampled (CFU/l)" were made (Table 36).

The preliminary data (Tables 36, 37 & 38 and Figure 11) showed that probably the most important single factor influencing the counts during this time was the presence or absence of cattle in the confinement housing units. Tables 36 and 37 summarize several weeks of comparable data during which the major independent variable was the presence or absence of cattle in the housing units. Counts are considerably less when cattle are absent and are in the same magnitude of counts in periods of human activity.

From the preliminary data, standard sampling locations were decided upon. Table 36 and Figure 6 show that samples at varying distances from the ditch east, south, north, and the field (100 meters west of unit) have the same order of total bacterial counts, indicating that the ditch itself did not have any profound effect on airborne counts more than a few meters away from the ditch, thus eliminating the need to continue sampling these areas. Instead, a 15-meter upwind and downwind sample was chosen for extramural sampling. This sampling procedure would warn of extraneous aerosol contamination or indict the ditch as a source.

Table 36. PRELIMINARY AEROSOL DATA TAKEN NOVEMBER 17, 1971 TO MAY 18, 1972 WHEN CATTLE WERE PRESENT

Viable Bacterial Aerosols at Different Sampling Sites (AGI Counts); on Plate Count Agar, 37°C, 24 hr. (colony forming units/liter)

	West vent*	East vent	West barn	East barn	Rotor	Field	Ditch	15 m E. of unit	15 m S. of unit	15 m N. of unit
Number of samples	18	18	32	29	20	19	12	9	10	1
Range CFU/1	2-399	1-126	8-1311	12-1208	11-192	0.05-10	3-158	0.1-2.8	0.12-7	0.06-1.3
Average CFU/1	39	21	234	261	46	1	50	1.4	1.4	0.5

* West Vent, east vent, west barn, and east barn counts are from samples taken at 30 and 100 cm from the ground; each sample represents the average of duplicates taken at that site.

This Table Separates the Above Sites into Sampled Heights, 30 and 100 cm

	West vent		East vent		West barn		East barn		Field	
	30 cm	100 cm	30 cm	100 cm	30 cm	100 cm	30 cm	100 cm	30 cm	100 cm
Number of samples	13	5	15	3	11	21	10	19	16	3
Range CFU/1	1-113	5-399	1-126	19-29	26-1311	6-1078	12-1208	22-1069	.05-2	.3-.6
Average CFU/1	20	89	21	24	323	188	326	230	1	0.4

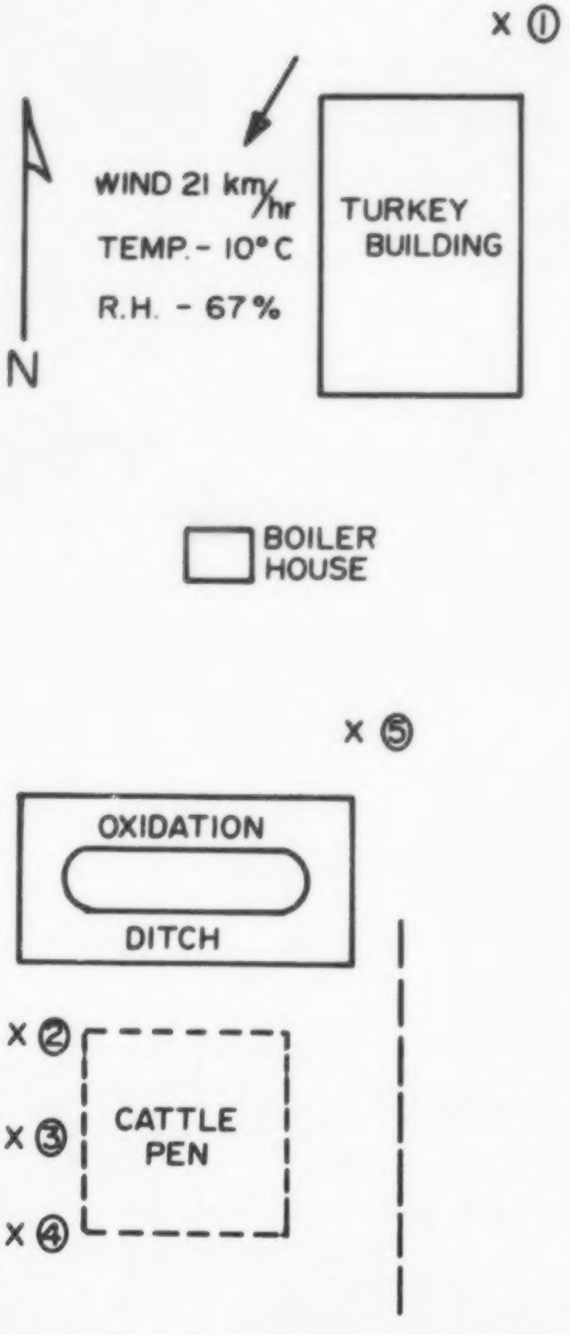
x ①
WIND 21 km/hr
TEMP. - 10° C
R.H. - 67%
N
TURKEY BUILDING
BOILER HOUSE
x ⑤
OXIDATION DITCH
x ②
x ③
x ④
CATTLE PEN

Figure 11. Air sampling locations at varying distances from oxidation ditch

Table 37. PRELIMINARY AEROSOL DATA TAKEN FEBRUARY 15 TO FEBRUARY 24, 1972 WHEN CATTLE WERE ABSENT

Viable Bacterial Aerosols at Different Sampling Sites (AGI counts); on Plate Count Agar, 37°C, 24 hr.
(colony-forming units/liter)

	West vent	East vent	West barn	East barn	Rotor	Field	Ditch	15 m E. of unit	15 m S. of unit	15 m N. of unit
Number of samples	7	6	12	11	2	7	3	3	4	2
Range	1-11	0.4-11	0.2-13	0.3-29	1-4	0.5-6	0.8-7	0.16-0.9	0.5-4	0.2-1.1
Average	6	3	4	8.3	2	2	3.1	0.59	1.6	0.6

Table 38. AIR SAMPLING AT VARYING DISTANCES FROM OXIDATION DITCH ON FEBRUARY 1, 1972
(colony-forming units/liter)

Figure 11 Location	Location description	CFU/1 on PCA
1	100 m N.E.	0.22
2	1.8 m S.W.	0.36
3	12 m S.W.	0.53
4	24 m S.W.	0.36
5	1.8 m N.E.	0.11

Table 36 indicates that there is a difference in counts due to varying elevations above ground, thus necessitating sampling at more than one elevation above grade.

The ditch samples, which were taken 100 cm above the surface of the ditch slurry (but beneath the ditch covering), showed that high counts were also directly dependent upon the presence of animals. This sampling was discontinued because the results would not indicate any relative degree of health hazard since animal or human exposure at that location is uncommon.

The need for consistency in sampling to assure statistical significance was recognized. To meet this need, the sampling procedure was set up to sample for the same length of time at each site and to make the same dilutions in each case. This consistency need was also shown in preliminary experiments. This is workable because in nearly all locations a countable number of total bacterial colonies appear. A sampling cart was constructed to transport pumps, hoses, samplers and electrical cords, greatly facilitating the sampling.

The early investigation of total aerobic bacteria present in the ambient air surrounding the ditch gave clues as to how to develop a technical sampling procedure. The detection of total aerobic bacteria did not indicate the amount of contamination from the ditch itself. The aerosol investigation of enteric indicator organisms (fecal Strep. and coliforms) was therefore added to the protocol.

Phase II, Finalized Sampling Program

Final Protocol --

At the end of May, 1972, the objectives specified in the preliminary stages of the project were completed. We had demonstrated the feasibility of an aerosol monitoring program and had learned enough about the technology, the environment, and the bioaerosol itself to design a sampling protocol whose results would be meaningful to both public health and air researchers.

The protocol followed was:

1. Sampling visits were to be made once every 7 ± 3 days.
2. Sampling would be at each of the following 7 sites:
   At the west vent on ditch housing
   At the east vent on ditch housing
   Inside the west housing unit
   Inside the east housing unit

Inside the rotor housing close to the turning rotor
Extramural site 15 meters upwind of the ditch
Extramural site 15 meters downwind of the ditch

3. At each site (except in the rotor housing) three samples were taken concurrently at 3 heights above the ground: 30 cm (ground level), 100 cm (cattle respiratory level), and 160 cm (human respiratory level). AGI nozzle openings were positioned to face toward any wind movement. Concurrent sampling devices are shown in Figures 12 and 13.

4. The rotor housing site was sampled by duplicate AGIs operating simultaneously at approximately 100 cm above the ditch slurry surface.

5. Each sample consisted of 62.5 liters (i.e., a 5-minute duration AGI sample) of air drawn into 30 ml of AGI collecting fluid (0.1% peptone and 0.01% Dow antifoam).

6. AGI samples were transported to the laboratory in a refrigerated thermal chest.

7. In the laboratory, the volumes of the AGI were made up to 30 ml with sterile diluent (to replace any evaporation loss) and 0.5-ml aliquots of the collecting fluids were plated in duplicate on prepared Plate Count Agar*, PCA (Tryptone-Glucose-Yeast Extract), on Levine's-EMB (L-EMB), and on m-Enterococcus* (m-Ent) agar. The aliquots were uniformly distributed over the medium surface with sterile, bent glass rods in a sterile hood. The plates were incubated at 37° C for 48 hours. Counts were reported as Colony-Forming Units per liter of air (CFU/1).

    a. Total CFU/1, all colonies on PCA were counted for computation.

    b. Coliform CFU/1; large, pigmented colonies on L-EMB that were gram negative rods and fermented lactose with production of gas on Triple Sugar Iron* (TSI) were counted for computation.

    c. Fecal Streptococci CFU/1; red colonies on m-Ent that were gram positive cocci, catalase negative, and that turned Bile Eschelin Agar* (BSA) black were counted.

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*Difco

Figure 12. Concurrent sampling device in barn

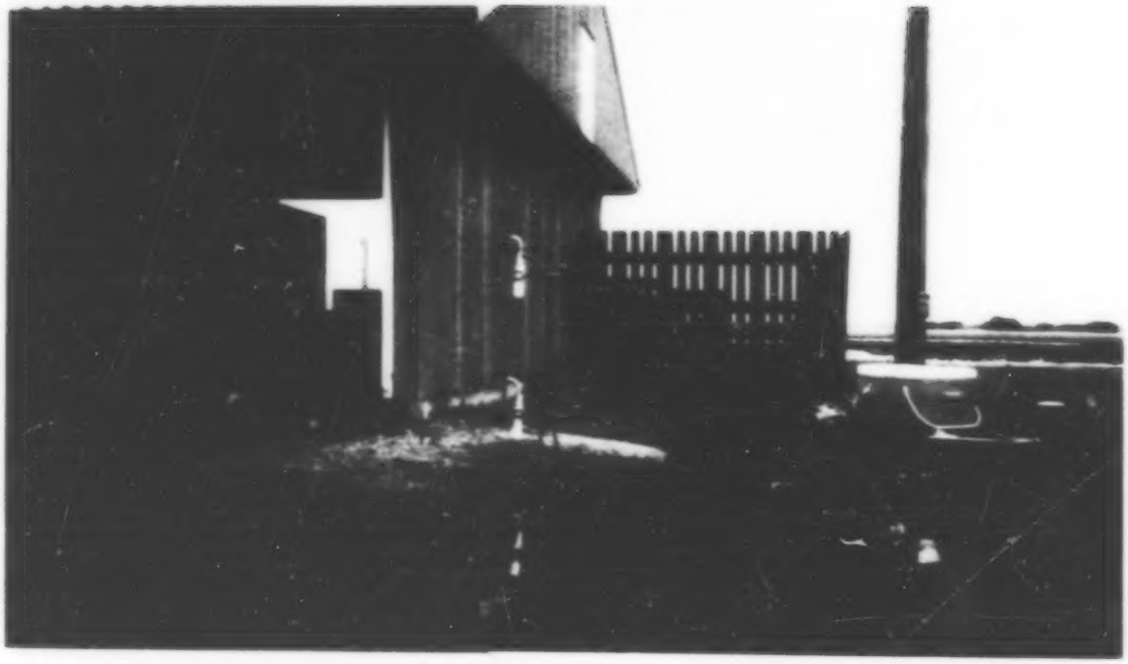

Figure 13. Concurrent sampling device outside of barn

Period of Sampling --

Using the standardized protocol described above, the routine monitoring program was initiated on May 25, 1972. From that date to June 13, 1973, 47 sampling visits were made to Rosemount. A full year cycle of climate was included during the sampling period.

RESULTS AND DISCUSSION

Data

The data from the 47 sampling visits are summarized in Tables 39 and 40.

Height of Sample

The air sampling at different heights showed a definite pattern. For example, at both east and west vents the 100-cm samplers gave, on the average, higher counts. This was probably due to the fact that

Table 39. AEROSOL DATA TAKEN WEEKLY MAY 25, 1972 TO JUNE 13, 1973 WHEN CATTLE PRESENT - ROSEMOUNT

Viable Bacterial Aerosols at Different Sites (AGI Counts) on PCA (Total), L-EMB (Coliform), and m-Ent (Fecal Streptococcus), 37°C, 48 hr. Counts Given in Colony-Forming Units/Liter (CFU/l)

Location	Height (cm)	Organism	Number Of samples	Total CFU/l	Range CFU/l	Average CFU/l	Average CFU/l 3 heights
West vent	30	Total	38	657	0-156	17	31
		Coliform	36	0	0-0	0	
		F. Strep.	37	1	0-1	0.03	
	100	Total	41	2145	2-194	52	
		Coliform	40	4	0-2	0.1	
		F. Strep.	40	2	0-1	0.05	
	160	Total	41	892	1-276	22	
		Coliform	39	3	0-3	0.08	
		F. Strep.	38	0	0-0	0	
East vent	30	Total	35	395	0-172	11	28
		Coliform	36	3	0-3	0.08	
		F. Strep.	34	4	0-4	0.12	
	100	Total	37	1856	0-268	50	
		Coliform	35	4	0-2	0.11	
		F. Strep.	35	10	0-5	0.29	
	160	Total	33	648	0-124	19	
		Coliform	32	0	0-0	0	
		F. Strep.	31	2	0-1	0.06	
West barn	30	Total	39	5132	1-580	132	117
		Coliform	34	14	0-8	0.41	
		F. Strep.	37	13	0-4	0.35	
	100	Total	37	4411	4-359	119	
		Coliform	35	8	0-5	0.23	
		F. Strep.	37	25	0-5	0.68	
	150	Total	41	4199	1-524	102	
		Coliform	39	5	0-2	0.13	
		F. Strep.	38	3	0-5	0.34	
East barn	30	Total	33	7991	9-1428	242	204
		Coliform	34	10	0-10	0.3	
		F. Strep.	34	31	0-14	0.9	
	100	Total	41	8814	4-837	215	
		Coliform	34	3	0-3	0.09	
		F. Strep.	38	26	0-5	0.7	
	160	Total	40	6418	9-794	160	
		Coliform	37	5	0-3	0.14	
		F. Strep.	37	47	0-33	1.3	

Table 39 (continued). AEROSOL DATA TAKEN WEEKLY MAY 25, 1972 TO JUNE 13, 1973 WHEN CATTLE PRESENT - ROSEMOUNT

Location	Height (cm)	Organism	Number Of samples	Total CFU/1	Range CFU/1	Average CFU/1	Average CFU/1 3 heights
Rotor		Total	40	2188	2-191	55	55
		Coliform	38	6.5	0-5	0.17	
		F. Strep.	37	5.5	0-2	0.15	
Upwind	30	Total	40	261	0-120	6.5	7
		Coliform	37	0	0-00	0	
		F. Strep.	37	0	0-00	0	
	100	Total	39	370	0-260	9.5	
		Coliform	38	0	0-0	0	
		F. Strep.	37	0	0-0	0	
	160	Total	38	181	0-29	4.8	
		Coliform	35	0	0-0	0	
		F. Strep.	36	0	0-0	0	
Downwind	30	Total	39	178	0-40	4.5	6
		Coliform	37	0	0-0	0	
		F. Strep.	37	1	0-1	0.05	
	100	Total	39	359	0-116	9.1	
		Coliform	38	0	0-0	0	
		F. Strep.	38	1	0-1	0.03	
	160	Total	38	137	0-41	3.6	
		Coliform	35	0	0-0	0	
		F. Strep.	35	0	0-0	0	

Table 40. AEROSOL DATA TAKEN AUGUST 9, 1972 TO SEPTEMBER 13, 1972 AND MARCH 1, 1973 TO MARCH 5, 1973 - CATTLE ABSENT

Location	Height (cm)	Organism	Number of samples	Total CFU/1	Range CFU/1	Average CFU/1	Average CFU/1 3 heights
West vent	30	Total	8	30	2-8	3.8	5.4
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	100	Total	8	55	2-18	6.9	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	160	Total	8	45	0-16	5.6	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
East vent	30	Total	8	194	0-182	24	12
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	100	Total	7	41	0-12	5.9	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	160	Total	8	34	0-10	4.2	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
West barn	30	Total	6	251	12-131	42	24
		Coliform	6	0	0-0	0	
		F. Strep.	6	0	0-0	0	
	100	Total	7	141	10-32	20	
		Coliform	7	0	0-0	0	
		F. Strep.	7	3	0-2	0.42	
	160	Total	7	86	6-25	12	
		Coliform	7	0	0-0	0	
		F. Strep.	7	0	0-0	0	
East barn	30	Total	8	26	0-7	3.2	10
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	100	Total	8	39	0-14	4.9	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	160	Total	8	170	0-148	21	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
Rotor		Total	8	130	8028	16	16
		Coliform	8	0	0-0	0	
		F. Strep.	8	1	0-0.5	0.12	

Table 40. AEROSOL DATA TAKEN AUGUST 9, 1972 TO SEPTEMBER 13, 1972 AND MARCH 1, 1973 TO MARCH 5, 1973 - CATTLE ABSENT

Location	Height (cm)	Organism	Number of samples	Total CFU/l	Range CFU/l	Average (CFU/l)	Average CFU/l 3 heights
Upwind	30	Total	8	6	0-6	0.75	1.6
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	100	Total	8	19	0-9	2.4	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	160	Total	8	13	0-6	1.6	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
Downwind	30	Total	8	14	0-6	1.8	1.7
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	100	Total	8	16	0-6	2	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	
	160	Total	8	10	0-2	1.2	
		Coliform	8	0	0-0	0	
		F. Strep.	8	0	0-0	0	

the 100-cm height is most directly in line with the vent. The other significant variation was in the two cattle housing units. In both barns the highest bioaerosol concentrations were at the 30-cm height and the lowest at the 160-cm height. This may mean that there is some ground contamination that does not reach the higher heights. The air is, however, being forced down through the slats and out due to the positive pressure ventilation system. The extramural counts both upwind and downwind showed the highest bioaerosol to be at the 100-cm height. In essence, however, the counts do not vary considerably from one height to another at any one site.

Housing Units

It is evident from the data that the air associated with the animal housing units harbors a rich bioaerosol. Counts approximately 100 to 200 total CFU/liter are between one and two orders of magnitude higher than those normally experienced by humans in non-agricultural occupations. It is equally evident that these high counts can be attributed almost entirely to the active, crowded presence of the livestock and not to the manure disposal system - the oxidation ditch method of extended aerobic treatment. When the steers were removed from the barn, within less than one hour, the bioload decreased to levels approximating those of the extramural environment (Table 40), even though the manure in the ditch was still being aerated by the rotor. This phenomenon is remarkably similar to the one already presented in Table 37 of the preliminary data. In essence, it may be said that the oxidation ditch contributes an insignificant number of microbes to the air when compared to the air when compared to the contribution of the living animals themselves.

Indicator Fecal Organisms

The numbers of indicator fecal bacteria enumerated in Tables 39 and 40 show that the high concentration is in the barns when animals are present. This is a strong indication that aerobic fecal contamination from the oxidation ditch, if present, is insignificant when compared to the contribution from the animals themselves.

Wetness of Manure

A wet condition of manure wastes, whether in the ditch slurry or the intramural surfaces of the housing units, inhibits aerosolization of bacteria. In contrast, dry matter of varying size particles becomes airborne by a slight amount of disturbance. For example, on one sampling day during preliminary investigations, animals were not present and therefore not wetting down the slats by urination. A worker had been in both barns producing dust by scraping off the accumulated residue on the slats. In both barns, counts were greater than 2,000 CFU/1, an unusually high level. On other days of the period when animals were absent and no scraping occurred, the counts averaged less than 10 total CFU/1.

Extramural Contamination

It was particularly gratifying to note that the extramural contamination levels, just a few dozen meters away from the barns, are considerably lower than expected. This implies that the barn aerosols do not persist for any significant distances or time periods extramurally.

Rotor Site

The fecal counts at the rotor site are most probably attributable to the ditch liquid, since the rotor is enclosed and open only to the ditch. This site has a microbioaerosol load which also fluctuates according to the presence or absence of animals. Table 41 indicates that even with the rotor off and thus no splashing, the counts in this area can be high. To further substantiate that the aerosol production was from the animals rather than the ditch slurry, sampling was done when the ditch contained only 6 cm of water and the rotor was stopped. Table 42 indicates that the counts are similar to conditions in Table 39 where the rotor is operating and the ditch slurry is higher.

Particulate Nuclei Theory

It would seem however, that due to the documented particulate nuclei theory (20) some of the ditch liquid would be aerosolized. Why then were there so few viable aerosols from the ditch? The three possible answers are: (1) bioaerosols created by the ditch are present but in a much lower concentration than is contributed by the animals and associated dust; (2) the physics of the manure slurry may inhibit the biological droplet nuclei production from the ditch due to the concentration of bacteria in the ditch; and (3) there is rapid die off of slurry resident microbes either in the slurry or in the air.

Comparison With Previously Reported Results

To evaluate the first possibility, other situations which produce droplet nuclei from a bacterial contaminate source were examined. Ladd (26), when sampling near a sewage pre-aeration basin, utilizing the Anderson-sieve sampler, was able to pick up tracer organisms that were poured down the laboratory drain approximately 8 km from the sewage plant. He was able to detect the organism at a maximum concentration of 0.008 CFU/l 5 hours after one pouring but none after the eleventh hour. Sewage has a much lower solids content than existent at all times in the oxidation ditch.

Randall (27) found in sampling air downwind from activated sludge units with an Anderson sampler that, on the average, 17 CFU/l of particle size less than 5 microns were found downwind of the operation (6% of which were Klebsiella).

Napolitano (28) studied concentrations of aerosols generated by activated sludge plants using EMB in an Anderson sampler. The highest count obtained was 8 CFU/l (no further selection was done). Further downwind at 15 and 30 meters, 0.18 CFU/l was found.

Albrecht (29) found when sampling with both a Wells centrifuge and midget impinger downwind from a trickling filter sewage treatment plant that very few coliform organisms were recovered but growth on nutrient agar showed recovery from 0.07 to 5 CFU/l at the edge of the

Table 41. BIOAEROSOL DATA TAKEN ON MARCH 28, 1973
WHEN DITCH HAD ONLY 6 cm WATER WITH ROTOR OFF
(colony-forming units/liter)

Location	Average CFU/l (total) over all 3 heights
West vent	40
East vent	50
West barn	80
East barn	144
Rotor	92
Upwind	2
Downwind	0.3

Table 42. BIOAEROSOL DATA TAKEN AT ROTOR SITE WHEN ROTOR OFF
(colony-forming units/liter)

Date	Average total CFU/l
July 25, 1972	48
March 28, 1973	92

trickling filter decreasing to 0.2 to 2.7 CFU/1 at a distance of 15 meters.

Adams (30) sampled the air with EMB in an Anderson sampler near a trickling filter. Without classifying bacteria any further than counting colonies on media, he found 19 CFU/1 at 15 meters downwind; 0.9 CFU/1 at 43 meters downwind and 0.003 CFU/1 at 1300 meters downwind.

In most of these instances, the bioaerosol load is similar (the extramural counts of the oxidation ditch producing the lowest counts), indicating that the production of bioaerosols of lung-penetrating size from contaminated liquid sources is not as high as dry sources - the Rosemount housing units for example. The following are also examples.

Cvjetanovic (31) monitored areas with a slit sampler and found total bacteria in surgery rooms to be 0.5-2 CFU/1, hospital corridors 6 CFU/1, animal houses 7-70 CFU/1, and gyms 1-13 CFU/1.

Duguid (32) found, when sampling for total bacteria with a slit sampler, that when a person is standing motionless in a room, 0.3 CFU/1 was produced; when there was slight activity, 4.3 CFU/1; vigorous activity produced 28 CFU/1; and undressing and dressing produced 56 CFU/1.

Examining the physics of the situation, that is:

$$\frac{10^6 \text{ bacteria}}{1 \text{ sq. cm}} = \frac{0.01 \text{ bacterium}}{1 \text{ sq. micron}}$$

it is doubtful that the concentration of ditch bacteria is high enough to be incorporated into droplet nuclei. There are approximately 10^6 total bacteria in a ml of ditch liquid. At this level, the chances of having a bacterial droplet nucleus 1 micron in size is 1 to 100.

Darlow (23), however, found that a tenfold reduction of total inoculum did not reduce aerosol concentration proportionately. When sampling with a slit sampler at seat level of a flushing toilet, he found approximately 1 CFU/1 when the bacterial concentration in the toilet was 5×10^5 cells/ml. He found that the number of bioaerosols produced was reduced threefold when the toilet lid was open vs. closed. If these results can be extrapolated to the ditch system, it seems that the ditch (being a closed area except at the rotor site where sampling was done 100 cm above the surface) would produce less than 1 CFU/1 and, therefore, is below the level of detection employed.

A literature review concerning the properties of the atmosphere which affect the airborne survival of bacteria pointed out that unknown factors could be influencing the counts. For example, Webb (33, 34) studied the effects of chemical additives on airborne cells and found that certain chemicals in the ditch could be influencing the survival. Chemical composition also affects the evaporation rate of a droplet, thus affecting the production of droplet nuclei (35).

Other studies showed that temperature and relative humidity (R.H.) affected the airborne survival rate of bacteria (36, 37). The data of this study was examined for patterns of counts which could be predicted due to meteorological variables, thereby modifying the temperature and R.H. if possible. The extramural atmosphere is, of course, not subject to modification. However, no pattern could be identified. Seasonal changes did not result in any change in counts. Table 43 illustrates the absence of seasonal meteorological influence upon counts. Wright (38) also found no correlation between counts and meteorological factors.

Table 43. INFLUENCE OF METEOROLOGICAL CHANGES ON EXTRAMURAL UPWIND COUNTS

Extreme Meteorological Conditions Give Same Counts

Date	Wind	Temp	RH%	Sky	Total CFU/l 30 cm	100 cm	160 cm
12/20/72	11 km/hr (SE)	-2 C	88	Overcast	0	0	0
5/16/73	30 km/hr (N)	9 C	48	Clear	0	0	0

Same Conditions Give Extreme Counts

Date	Wind	Temp	RH%	Sky	30 cm	Total CFU/l 100 cm	160 cm
4/18/73	24 km/hr (SE)	20 C	48	Clear	0	0	0
5/30/73	13 km/hr (SW)	21 C	49	Clear	9	22	29

Calculation of the average temperature and R.H. in the barns shows that, for the west barn the average temperature was 19° C and R.H. 73%, while for the east barn it was 17° C, 75% R.H. This small difference then does not account for the higher concentration of bacteria in the east barn. This higher concentration is believed to be due to the only known difference between barns, the slat operation, allowing less air to pass through and, thus, less mass movement of air containing aerosols from the housing unit.

Gaseous Odor Effects

One contributable alteration was the noticeable effect of a gaseous odor upon counts. This odor of some sort of fuel in the west barn was noticed on three days of sampling. Table 44 shows the reduced bioaerosol counts in the west barn when the odor was present, but the counts in other areas were near average. The odor was determined to be from preservative-treated lumber used to repair the inside of the housing unit.

Table 44. BIOAEROSOL DATA TAKEN WHEN WEST BARN SMELLED OF FUEL ODOR
(average total CFU/1 over all 3 heights)

Location	March 1, 1973	March 16, 1973	March 23, 1973	Average
West vent	8	7	4	6
East vent	25	4	5	11
West barn	47	7	6	20
East barn	153	553	20	242
Rotor	ND	ND	2	2
Upwind	2	0	ND	1
Downwind	8	3	ND	5.5

Health Hazard Evaluation

Essentially, the Rosemount study shows that the public health hazard associated with the aerosols from the ditch is much less than that associated with the animal housing operation.

Comparison of Rosemount to Other Waste Treatment Facilities

Other University of Minnesota cattle waste treatment facilities were in operation at Morris, Minnesota. These operations afforded the opportunity to sample different systems to further evaluate microbioaerosol production in a beef animal facility.

At Morris, air samples were taken with the AGI inside three different animal housing units and at the exhaust air outlet venting pit area of one of the enclosed slatted animal housing units. One of the housing units sampled was roofed but open on one side; the animals were housed on slats above a standing quiescent anaerobic pit (open-slat). Another housing unit sampled was open on one side and the animals were bedded with hay (open-bedded). The third unit was a warm, enclosed, slatted floor beef unit with capacity for 70 animals. A quiescent anaerobic pit collects the waste below the slats. The temperature, R.H. and ventilation are similar to the Rosemount facility. The samples were taken at three heights consecutively (30, 100, 160 cm) except at the vent where the sampling was directly in front of the vent. The samples were analyzed for total, coliform, and fecal streptococci organisms in the same manner as Rosemount.

Table 45 gives the bioaerosol counts of the Morris beef animal facility. The results are remarkably similar to those taken at Rosemount. Extramural counts are zero CFU/1 and counts taken at the vent are 48 CFU/1. The slatted housing units, both open and closed, show bioaerosol counts decrease as the elevation of the sample increases. The open bedded counts are believed to be lower than the other housing units because the wind was blowing from the AGIs toward the animals and hay. The comparison sampling of these other animal housing units substantiates the counts of the Rosemount area. Note that the pit holding tanks were not aerated, thus eliminating droplet nuclei formation.

From the various sampling done, the expected microbioaerosol total and indicator counts from the oxidation ditch manure disposal system and the animal confinement areas of such systems have been revealed. However, the public health hazard can at this time only be assessed by comparison with other waste management operations. For example, if it takes a certain number of bacteria to infect, a comparison can be made to the infection danger of other systems by evaluating the bioload of fecal bioaerosols in those systems and indirectly assess the hazard. For this reason and to compare a traditional system utilizing our protocol, lab, and personnel, a program of aerosol sampling at a St. Paul Campus dairy barn was conducted from February 21, 1973, through June 11, 1973. The results of this investigation gave another parameter with which to determine the relative public (occupational) health

Table 45. MORRIS SAMPLING WITH AGI
(colony-forming units/liter of air sampled)

Location	30 cm			100 cm			160 cm		
	Total	Colif.	F.Strep.	Total	Colif.	F.Strep.	Total	Colif.	F.Strep.
Upwind	0	0	0	0	0	0	ND	ND	ND
Open-slat	156	0	3	72	0	2	12	0	0
Open-bedded	18	0	0	31	0	0	59	0	0
Enclosed-slat	175	0	2	83	0	3	40	0	0
Exhaust-vent	48	0	0	ND	ND	ND	ND	ND	ND

Sampling date: March 13, 1973
Extramural conditions: 13° C, 78% RH
Intramural conditions: 18° C, 55% RH
(enclosed-slat)
Partly cloudy sky

hazard of an oxidation ditch to traditional methods of waste handling utilized in animal husbandry. This study continued on a weekly basis to obtain microabioaerosol data for comparison with the Rosemount information. The dairy barn manure disposal is by gutter cleaner, shovel, washing when required, and sweeping. Continuously operating fans exhaust air from the barn. Samples were taken in the center of the alleyway between the two rows of producing cows. The samples were analyzed for total plate count, coliform, and fecal Streptococci in the same manner as at Rosemount.

Samples in the dairy barn were taken consecutively starting at the lowest level (30 cm) and proceeding to the highest (160 cm) at a single location, once in the morning and once in the afternoon, in order to monitor variable conditions. In the morning, the animals were quiet; in the afternoon the animals were eating and the floors were being swept. Table 46 shows that the counts are considerably higher during the afternoon activity. The counts show that the animals and workers in the area are exposed to comparable numbers of total and fecal bioaerosols to the Rosemount area. This is and has been a traditional system that has withstood any changes of its environment due to a public health necessity.

This comparative study and the previously given studies have given us information with which to evaluate the oxidation ditch waste disposal system to be non-hazardous to public health as far as the potential contamination of microbioaerosols from the oxidation ditch.

Table 46. DAIRY BARN SAMPLING

Aerosol Data Taken Weekly February 21, 1973 - June 11, 1973
When Cattle Present

Viable Bacterial Aerosols During Different Conditions (AGI Counts) on PCA (total), L-EMB (coliform), and m-Ent (Fecal Streptococcus), 37°C, 24 hr. Counts Given in Colony-Forming Units/Liter (CFU/l)

		Quiet conditions			Sweeping and/or eating			Compilation of all 3 heights, both conditions
Height above floor	Organism	Number of samples	Range CFU/l	Average CFU/l	Number of samples	Range CFU/l	Average CFU/l	
30 cm	Total	14	38-266	112	12	75-826	294	Average Total CFU/l: 196
	Coliform	12	0-0	0	12	0-3	0.25	
	F. Strep.	14	0-3	0.43	12	0-5	0.67	
100 cm	Total	15	22-222	92	12	57-1168	370	Average Coliform CFU/l: 0.08
	Coliform	13	0-0	0	12	0-0	0	
	F. Strep.	15	0-2	0.2	12	0-3	0.67	
160 cm	Total	15	40-240	107	12	78-820	261	Average F. Strep. CFU/l: 0.49
	Coliform	13	0-1	0.15	12	0-1	0.08	
	F. Strep.	15	0-2	0.4	12	0-3	0.67	

SECTION VI

MODEL B OXIDATION DITCH STUDIES

INTRODUCTION

The model oxidation ditch, MOD-B, was situated in the Agricultural Engineering Waste Management laboratory to conduct studies on the degradation of waste. Field-scale oxidation ditches exposed to the constantly changing environment have been studied extensively by Moore et. al. (39). More work was needed to study the degradation of beef manure under more closely controlled conditions.

OBJECTIVE

The objective of the study was to determine relationships between temperature and rotor speed and the breakdown of the beef waste under controlled conditions simulating the field oxidation ditch at Rosemount.

MATERIALS AND METHODS

Model B Oxidation Ditch

The Model-B oxidation ditch was a modified version of the model used by Diesch and Allred (40) during the initial Public Health Service Grant Project. A steel, four-wheeled cart supported the 1/10th scale plexiglas model ditch on a steel frame. A variable speed reducer provided the power through a chain drive to the brush rotor in one end of the ditch. To achieve the objective in the 2-year period instead of the planned 3-year period, a third model ditch (MOD-C) was constructed and used during 1973.

Environmental Control Chamber

To provide a controlled temperature environment and constant humidity control, a control chamber was purchased and installed. Made up of prefabricated sections of aluminum-clad styrofoam, the chamber was easily constructed in the Agricultural Engineering Waste Management Laboratory. The chamber is approximately 4 meters by 3 meters and 3 meters high. A water chiller unit was mounted on the roof and a diffuser with a fan mounted inside the chamber. The temperature was controlled by a thermostat which regulated a Jabsco pump that moved water through a flexible hose from the water chiller to the diffuser.

Experimental Procedure

The Environmental control chamber was adjusted to one of the three temperatures for a specific test. Table 47 shows the various combinations of rotor speed and temperature investigated. Next, the rotor speed was adjusted and tested using a hand hold tachometer and a stop watch. The ditch in the chamber was then filled with tap water to a depth of 4.5 cm (1 3/4"). With the rotor operating, fresh beef cattle feces were added until a level of approximately 0.25% total solids was reached. After operating for several days to get a steady population of microorganisms the tests were started. Fresh feces were added each day during the work week. Wet chemical tests were performed twice a week.

Table 47. EXPERIMENTS CONDUCTED ON MODEL B OXIDATION DITCH

Dates	Run	Rotor Speed (RPM)	Temperature (°C)
Shakedown run only	#1		
5-31-72 to 7-7-72	#2	180	20°
7-17-73 to 8-28-73	#3	380	20°
9-1-72 to 10-17-72	#4	275	20°
7-20-73 to 8-28-73	#5	180	2°
3-5-73 to 4-13-73	#6	275	2°
4-19-73 to 5-25-73	#7	380	2°
5-23-73 to 6-21-73	#8	275	10°
6-14-73 to 7-19-73	#9	380	10°
6-21-73 to 8-9-73	#10	180	10°

Laboratory Procedures

The procedures described in Standard Methods for the Examination of

Water and Waste Water, 13th Edition, 1971, were used to analyze COD, solids, total nitrogen, and phosphate. Devarda's Alloy Method was used in the determination of nitrate. Carbon and hydrogen were determined using a Coleman model 33 carbon hydrogen analyzer. Dissolved oxygen was monitored using a Yellow Springs Instrument model 54 oxygen meter and pH was monitored with a Heath model EU-20-31 pH recorder and electrometer.

RESULTS AND DISCUSSION

Data

The data from tests 2 through 10 on the model oxidation ditch B are presented in tables 48 through 56 following this section.

Dissolved Oxygen Levels in the Ditch Slurry

One of the primary benefits derived from using an oxidation ditch is that aerobic conditions are maintained as the animal waste is degraded. Odor-free operating conditions are a result of this aerobic degradation. The dissolved oxygen levels indicate the amount of oxygen available for the aerobic microorganisms. At no time during any of the tests did the dissolved oxygen level even approach zero. In fact, during the most severe test, at Summer temperature 20° C and slow rotor speed (180 RPM), the D.O. reached 2.0 mg/1 on one occasion. During cold-weather tests at 2° C, the D.O. was normally above 12 mg/1.

Evaporation From the Ditch

There was need to continually add water to the ditch to maintain the desired level of slurry in the ditch. This was expected because the aerobic decomposition products include water vapor. In the field ditch, leaking waters and spilled water provided additional liquid which was not added in the laboratory study. Also, it was observed that the storage volume in field oxidation ditches did not build up as fast as expected, due to evaporation. Thus, a slight benefit was gained as less storage space was needed for extended detention with the oxidation ditch when compared to anaerobic, liquid manure tanks.

pH Values

The pH of the slurry was either neutral or slightly basic during all of the tests. The aerobic process does not promote formation of acid as is the case with the anaerobic process of degradation. Very little fluctuation of pH occurred during the tests, from which it could be generalized that the system was well buffered also.

Total Solids Content

As would be expected, the total solids (TS) content of the slurry increased as the individual test progressed. Because solids were being added and the only solids removed were those in test samples, the TS should continually increase. Also, because the solids added are not all volatile (able to be biologically degraded) but partly ash, the continual increase in TS was expected. Storage of the solids is necessary and also affects the ability of the rotor in transferring oxygen to the slurry. As a result, lower dissolved oxygen levels were observed as the test would progress to higher solids content levels. For example, in test number 2 on May 31, 1972, the TS was 9,232 mg/l and the D.O. was 6.9 mg/l. In contrast, on July 7, 1972, the TS was 12,562 mg/l and the D.O. had dropped to 4.35 mg/l. The tests did not continue as long as some which have been conducted in the field oxidation ditch at Rosemount. The TS contents in the field ditch have been allowed to reach at least 60,000 mg/l on several occasions. The dissolved oxygen has approached zero and the movement of the ditch slurry slowed considerably. The model ditch reacted within the scope of the experiment, as did the field ditch. Total Solids is a prime indication of physical load being placed on the moving rotor as the rotor lifts, aerates, and propels the slurry in the raceway-shaped tank. When TS are elevated, more energy from the rotor is expended moving the slurry and less is available to oxygenate the slurry. The model ditch was able to maintain aerobic conditions in the slurry at the loading levels imposed during these tests.

Nitrate in the Slurry

No conclusions could be derived from the nitrate tests performed. The levels varied from no measurable amount to 175 mg/l.

Phosphate in the Slurry

Phosphate should be conserved in a closed system such as the oxidation ditch. Phosphorus is not in volatile compounds normally associated with bacterial degradation. The phosphate data have an increasing trend in all tests with the dips probably related to sampling and analytical errors. When the slurry is returned to the land at the time the ditch contents are removed, the phosphate will again be in the productive part of the phosphate cycle.

Total Volatile Solids

The total volatile solids (TVS) were affected by temperature. At the lower temperature, 2° C, the TVS were a larger fraction of the waste because biological breakdown was progressing at a much slower rate.

Total Carbon

In each test, total carbon increased as more material was added. The increase followed that of TVS as would be expected, since both are indicative of organic matter in the slurry. Carbon levels were higher in the test at colder temperatures, which indicates slower degradation and buildup of organic matter.

Chemical Oxygen Demand

The chemical oxygen demand test (COD) is a measure of the waste strength in terms of oxidizable matter. Figures 14, 15, and 16 show COD vs. Time for the various RPMs at constant temperature on each figure. At cold temperatures, the organic matter builds up at a faster rate than at the 20° C temperature. At 20° C, RPM has no observable effect on breakdown rate as shown by the parallel trend of COD buildup.

At 10° C an acclimatization effect with the COD buildup trend rising sooner at 275 RPM occurred, then the 380 RPM, and, finally, the 180 RPM. This may be due to start-up problems and bacteria population in the ditch. Overall breakdown is less as rotor speed is decreased.

At 2° C, the least amount of breakdown was occurring. Population growth is slower at low temperatures so the breakdown is as expected. Rotor speed does not show a noticeable effect because there is an abundance of oxygen in the cold water to satisfy the aerobic microorganisms.

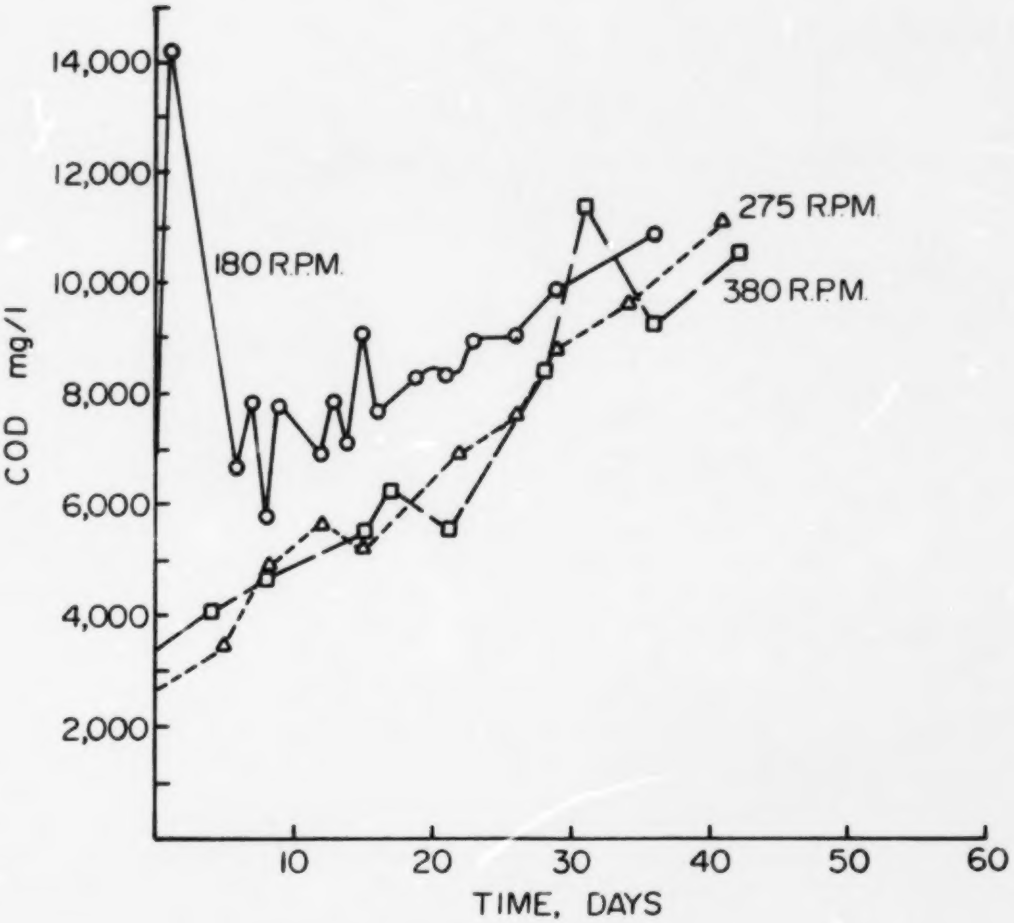

Figure 14. Chemical oxygen demand vs. time at 20° C for 3 RPMs

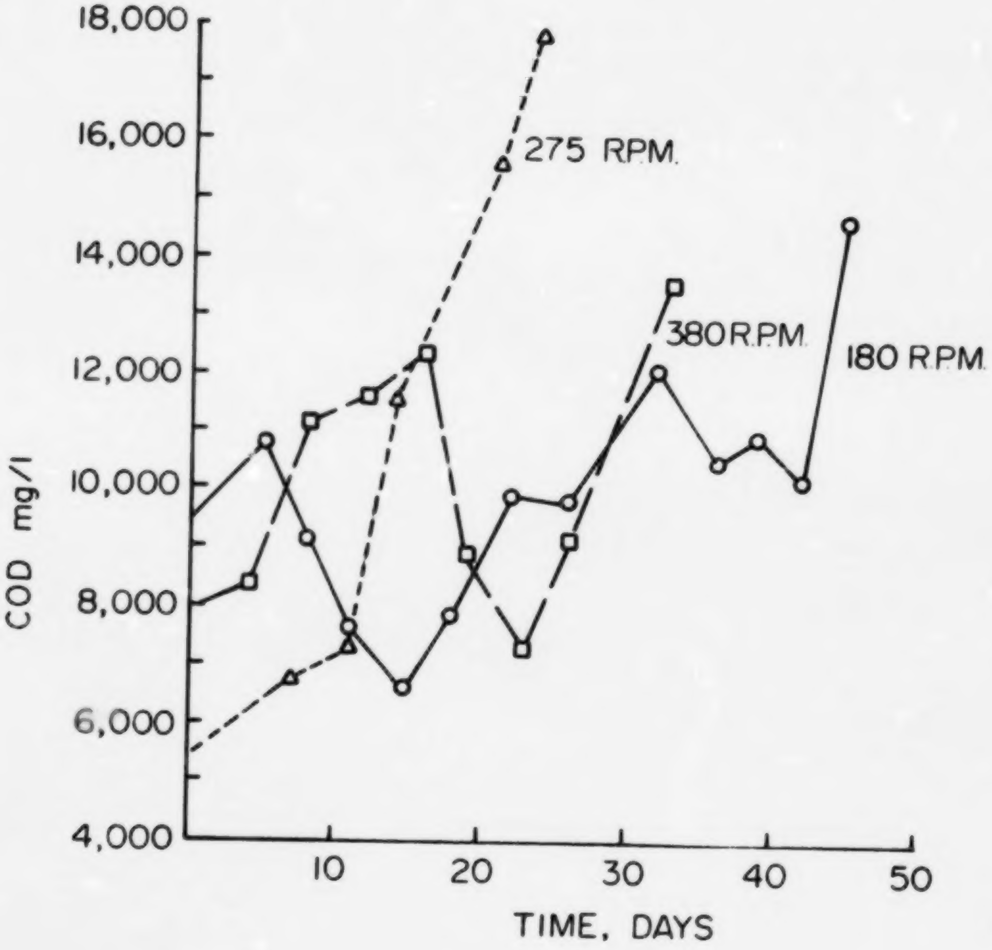

Figure 15. Chemical oxygen demand vs. time at 10° C for 3 RPMs

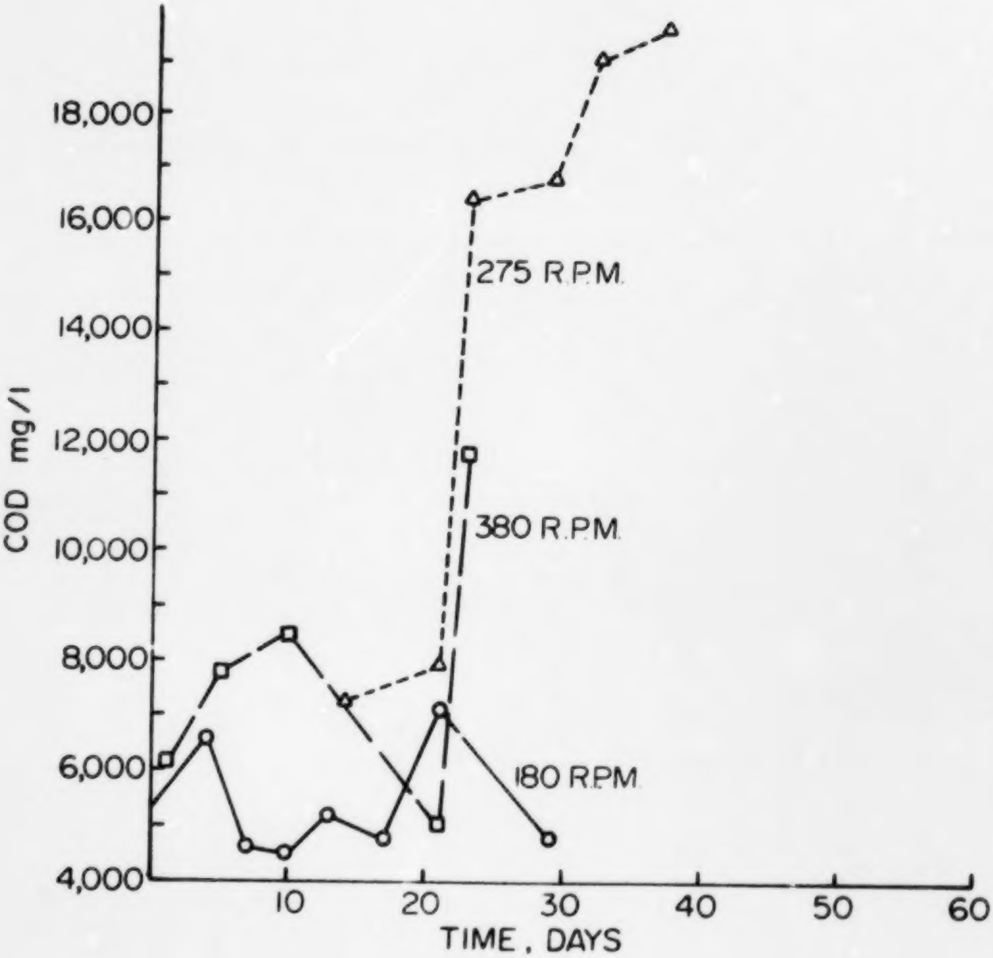

Figure 16. Chemical oxygen demand vs. time at 2° C for 3 RPMs

Table 48. RUN NUMBER 2, ROTOR SPEED 180 RPM, TEMP. 20° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitrogen (mg/1)	Car-bon (%)	Hydro-gen (%)
5-31-72	130.0	8	9,232	5,747	8.5	6.9	6,784	813	175.0	384.3	27.58	4.32
6-1-72	137.7	4	8,243	5,315	8.5	7.1	6,762	1,056	ND	377.4	27.72	4.39
6-2-72	132.4	4	8,360	5,593	8.1	4.6	14,221	ND	41.72	140.0	29.83	4.54
6-5-72	120.0	ND	7,236	4,518	8.7	7.4	6,646	ND	15.85	350.3	29.55	4.51
6-6-72	130.6	8	8,140	5,322	8.3	6.6	7,805	590	ND	374.6	30.36	4.67
6-7-72	128.8	4	6,449	4,111	8.8	4.0	5,752	437	55.0	271.6	28.49	4.45
6-8-72	135.3	4	6,865	4,417	8.1	3.9	ND	237	8.24	259.5	29.88	4.56
6-9-72	131.6	4	8,422	5,782	8.0	8.3	7,770	553	ND	336.5	31.13	4.88
6-12-72	151.0	4	9.669	6,610	8.15	6.7	7,126	581	ND	138.2	32.82	4.87
6-13-72	143.5	4	9,844	6,835	8.15	5.0	7,918	ND	ND	274.0	32.20	5.15
6-14-72	164.6	ND	ND	ND	ND	ND	7,170	586	ND	ND	ND	ND
6-15-72	143.7	4	10,255	7,149	8.05	4.3	9,042	513	3.66	195.0	32.78	5.25
6-16-72	130.1	4	11,618	8,372	8.25	5.9	7,548	ND	1.93	450.0	35.30	5.43

Table 48 (continued). RUN NUMBER 2, ROTOR SPEED 180 RPM, TEMP. 20° C

Date	Waste added (g)	H_2O added (l)	Total solids (mg/l)	Total volatile solids (mg/l)	pH	Dis-solved oxygen (mg/l)	Chemical oxygen demand (mg/l)	PO_4 (mg/l)	NO_3 (mg/l)	Total nitrogen (mg/l)	Car-bon (%)	Hydro-gen (%)
6-19-72	143.3	4	10,063	6,950	8.05	6.4	8,256	405	5.36	446.0	32.89	5.13
6-20-72	138.0		11,072	8,228	8.4	5.2	8,423	460	6.81	502.0	34.18	5.37
6-21-72	143.0	4	10,993	7,897	8.5	4.8	8,258	413	3.23	525.0	33.17	5.33
6-22-72	143.5	ND	11,124	8,180	8.51	5.3	8,309	558	ND	482.0	33.80	5.24
6-23-72	145.3	4	10,499	7,469	8.50	5.9	8,987	663	5.51	506.0	32.46	5.18
6-26-72	145.5	4	10,870	7,727	8.50	6.2	9,026	512	5.76	336.0	33.60	4.99
6-27-72	145.1	ND	ND	ND	8.35	5.2	ND	ND	ND	ND	ND	ND
6-29-72	144.0	4	13,083	9,886	8.5	6.0	9,853	633	7.93	535.0	ND	ND
6-30-72	132.7	4	ND	ND	8.4	3.0	ND	ND	ND	ND	ND	ND
7-3-72	145.0	4	12,957	9,850	ND	6.0	10,614	650	6.49	547.0	35.99	5.35
7-5-72	147.4	ND	ND	ND	8.3	2.0	ND	ND	ND	ND	ND	ND
7-6-72	139.2	ND	12,562	9,383	8.4	4.35	10,736	695	8.30	561.0	36.09	5.34
7-7-72	148.8	ND	ND	ND	8.4	5.9	ND	ND	ND	ND	ND	ND

Table 49. RUN NUMBER 3, ROTOR SPEED 380 RPM, TEMP. 20° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
7-17-73	131.9	8	3542	2623	8.4	8.3	3487	185	10.1	229	34.45	5.50
7-18-73	142.4	0	ND	ND	8.4	7.6	ND	ND	ND	ND	ND	ND
7-19-73	131.4	4	ND	ND	ND	8.0	ND	ND	ND	ND	ND	ND
7-20-73	146.3	3	4534	3202	8.3	ND	4016	185	11.7	321.6	35.68	5.63
7-21-73	133.4	2	ND	ND	8.35	7.3	ND	ND	ND	ND	ND	ND
7-24-73	147.1	4	5040	3865	8.45	7.7	4658	155	11.62	297	37.62	5.34
7-25-73	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-26-73	143.8	8	ND	ND	8.5	ND	ND	ND	ND	ND	ND	ND
7-27-73	141.2	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-28-73	0	0	5496	4137	ND	ND	ND	235	ND	319.2	36.54	5.16
7-31-73	129.0	8	ND	ND	ND	ND	ND	ND	ND	325.13	ND	ND
8-1-73	131.2	4	5277	3958	6.45	8.3	5538	256	19.04	308.8	35.95	5.42
8-2-73	153.7	1	ND	ND	6.3	8.0	ND	ND	ND	ND	ND	ND
8-3-73	141.8	4	6677	5215	6.2	7.9	6246	219	ND	401.2	39.07	5.61

Table 49 (continued). RUN NUMBER 3, ROTOR SPEED 380 RPM, TEMP. 20° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
8-4-73	127.0	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-7-73	0	0	5514	4060	6.4	ND	5570	136.3	ND	ND	ND	ND
8-8-73	143.3	2	ND	ND	8.50	7.9	ND	ND	ND	ND	ND	ND
8-9-73	146.7	2	ND	ND	8.55	7.9	ND	ND	ND	ND	ND	ND
8-10-73	145.7	2	ND	ND	8.42	7.6	ND	ND	ND	ND	ND	ND
8-11-73	127.3	2	ND	ND	8.5	7.5	ND	305.0	ND	ND	ND	ND
8-14-73	145.2	9	8659	6646	6.4	7.5	8472	152.2	ND	466	36.20	5.48
8-15-73	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-16-73	141.7	4	ND	ND	8.8	7.0	ND	ND	ND	ND	ND	ND
8-17-73	87.4	2	9392	7354	ND	7.3	11444	170	ND	452	38.81	5.38
8-18-73	0	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-21-73	149.3	4	9011	6816	8.6	8.3	ND	152	ND	490.7	36.93	4.96
8-22-73	146.9	6	8751	6572	ND	7.4	9273	168	12.2	ND	38.24	5.18
8-23-73	148.3	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-25-73	147.0	6	11060	8773	8.6	7.0	9841	270	8.9	502	38.75	5.34
8-28-73	140.4	4	10900	8461	8.65	7.8	10528	360	11.17	552	38.65	5.22

Table 50. RUN NUMBER 4, ROTOR SPEED 275 RPM, TEMP. 20° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total Volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total Nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
9-1-72	341	39	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9-4-72	84.3	10	1714	ND	ND	ND	ND	ND	ND	ND	ND	ND
9-6-72	144.2	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9-7-72	148.3	2	2491	1960	ND	ND	2588	ND	ND	131.9	41.20	5.96
9-8-72	149.2	4	ND	ND	8.45	7.4	ND	ND	ND	ND	ND	ND
9-11-72	145	6	3639	2864	8.50	7.5	3488	ND	3.30	194	39.12	5.96
9-12-72	137.3	4	ND	ND	8.50	8.5	ND	ND	ND	ND	ND	ND
9-13-72	146.4	2	ND	ND	8.5	7.8	ND	ND	ND	ND	ND	ND
9-14-72	125.1	2	5173	4219	ND	ND	4924	ND	3.02	264	41.01	5.89
9-18-72	126.7	6	5518	4388	8.6	6.8	5687	185	ND	313	40.49	5.93
9-20-72	133.3	4	ND	ND	8.5	6.1	ND	ND	ND	ND	ND	ND
9-21-72	145.7	2	5955	4740	8.55	8.0	5194	ND	2.77	329	41.34	5.94
9-22-72	139.1	4	ND	ND	ND	ND	ND	367	3.44	ND	ND	ND

Table 50 (continued). RUN NUMBER 4, ROTOR SPEED 275 RPM, TEMP. 20° C

Date	Waste added (g)	H_2O added (l)	Total solids (mg/l)	Total volatile solids (mg/l)	pH	Dis-solved oxygen (mg/l)	Chemical oxygen demand (mg/l)	PO_4 (mg/l)	NO_3 (mg/l)	Total nitro-gen (mg/l)	Car-bon (%)	Hydro-gen (%)
9-27-72	140.8	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9-28-72	129.7	4	6499	5041	ND	ND	6904	ND	ND	304	39.67	5.76
9-29-72	156.6	4	ND	ND	8.5	6.2	ND	350	6.44	ND	ND	ND
10-2-72	175.6	8	7985	ND	ND	ND	7555	ND	ND	418	37.08	5.80
10-3-72	170.8	4	ND	ND	ND	ND	ND	ND	ND	417	ND	ND
10-5-72	200.3	4	8062	6182	8.5	7.3	8734	400	5.68	ND	39.97	5.87
10-6-72	149.2	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10-10-72	262.6	8	8999	6961	ND	ND	9603	511.9	3.36	313	39.81	5.69
10-11-72	205.0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10-12-72	196	4	10294	8117	8.5	6.4	9940	587.2	ND	504	39.66	5.51
10-13-72	180.8	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10-16-72	170.5	4	11139	8820	ND	ND	ND	ND	3.86	ND	39.11	5.29
10-17-72	180.3	4	ND	ND	8.55	6.8	11020	598.6	ND	ND	ND	ND

Table 51. RUN NUMBER 5, ROTOR SPEED 180 RPM, TEMP. 2° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
7-20-73	382.0	19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-23-73	0	0	11230	9685	7.32	ND	19062	1319	NMA	542	44.789	6.260
7-24-73	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-26-73	0	6	3470	3030	7.46	ND	12155	608.5	NMA	168	45.755	6.054
7-31-73	0	4	4180	3793	7.74	15.5	5388	19.2	NMA	213	ND	ND
8-1-73	109.4	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-2-73	106.1	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-3-73	0	1	3450	3070	7.88	12.3	6554	649	NMA	246	44.454	6.630
8-6-73	166.6	3	3600	3040	8.05	12.0	4630	131	NMA	235	44.735	6.139
8-7-73	130.5	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-8-73	126.9	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-9-73	106.16	4	5817	5130	7.46	11.9	4596	212.5	NMA	343	45.171	6.104
8-10-73	109.56	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 51 (continued). RUN NUMBER 5, ROTOR SPEED 180 RPM, TEMP. 2° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
8-13-73	121.9	4	5376	4737	7.96	12.4	5171	259	NMA	287	ND	ND
8-15-73	124.6	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-16-73	109.6	0	5300	4533	7.43	13.0	4744	238	ND	306	44.401	6.464
8-20-73	137.5	0	3870	3410	7.89	12.0	7126	322	NMA	382	41.230	5.228
8-24-73	109.3	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-27-73	0	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8-28-73	0	0	9370	8250	7.23	13.5	4816	1646	NMA	440	43.204	5.650

Table 52. RUN NUMBER 6, ROTOR SPEED 275 RPM, TEMP. 2° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
3-5-73	0	0	6303	5396	ND	ND	ND	377	ND	ND	ND	ND
3-7-73	170.6	2	7343	6316	ND	ND	ND	394	ND	367	ND	ND
3-9-73	161.8	2	ND	ND	ND	ND	ND	438	ND	340	ND	ND
3-20-73	158.5	15	6791	5790	8.3	12.0	7236	379	NMA	384	ND	ND
3-21-73	164.1	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-22-73	0	0	ND	ND	8.3	12.8	ND	39.48	ND	350	ND	ND
3-23-73	153.4	2	8032	6822	ND	ND	ND	ND	ND	ND	ND	ND
3-27-73	156.9	2	7976	6703	8.2	11.9	7968	447.6	NMA	405	ND	ND
3-29-73	159.2	2	ND	ND	8.3	ND	16362	486	ND	442.6	ND	ND
4-4-73	149.1	3	10304	9156	ND	ND	ND	ND	ND	494.89	ND	ND
4-5-73	161.5	3	10339	8882	ND	ND	16711	557.3	NMA	541	ND	ND
4-9-73	150	3	ND	ND	ND	ND	18945	ND	ND	ND	ND	ND
4-10-73	136	3	11313	9672	ND	ND	ND	586.9	ND	635	ND	ND
4-13-73	0	0	ND	ND	ND	ND	19585	583.2	ND	ND	ND	ND

Table 53. RUN NUMBER 7, ROTOR SPEED 380 RPM, TEMP. 2° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
4-19-73	1633	60	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-24-73	136.6	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-26-73	153.2	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-27-73	157.8	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5-1-73	155.7	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5-2-73	147.9	9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5-3-73	0	0	6820	6017	7.85	ND	ND	ND	ND	387.3	40.345	6.236
5-4-73	155.7	3	ND	ND	ND	ND	6102	ND	ND	ND	ND	ND
5-7-73	0	0	8054	7152	ND	ND	7738	258	ND	325.6	ND	ND
5-9-73	150.0	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5-10-73	148.6	3	8027	7038	ND	ND	8478	308	ND	452.0	ND	ND
5-14-73	152.4	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5-15-73	158.5	3	9253	8057	ND	ND	ND	422	ND	477.1	20.725	3.880

Table 53 (continued). RUN NUMBER 7, ROTOR SPEED 380 RPM, TEMP. 2° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
5-18-73	150.0	4	ND	ND	ND	ND	ND	2873	ND	421.9	24.039	3.335
5-23-73	114.7	4	9613	8392	8.20	NMA	5090	263.4	NMA	428.8	41.52	6.32
5-25-73	100	32	12240	1283	7.97	ND	11885	404.2	NMA	506.5	37.937	6.054

Table 54. RUN NUMBER 8 ROTOR SPEED 275 RPM, TEMP. 10° C

Date	Waste added (g)	H_2O added (l)	Total solids (mg/l)	Total volatile solids (mg/l)	pH	Dis-solved oxygen (mg/l)	Chemical oxygen demand (mg/l)	PO_4 (mg/l)	NO_3 (mg/l)	Total nitro-gen (mg/l)	Car-bon (%)	Hydro-gen (%)
5-23-73	0	0	5496	4646	7.41	ND	4127	277.2	NMA	342.5	37.943	6.055
5-25-73	100	4	8147	1061	7.59	ND	10372	362	NMA	436.2	ND	ND
5-29-73	0	8	ND	ND	8.14	ND	5322	270.2	ND	322.2	38.32	5.93
5-30-73	113.6	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5-31-73	106.4	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-1-73	140.1	2	6398	1042	7.70	ND	ND	347	NMA	474	ND	ND
6-4-73	157.3	4	5400	899	8.13	ND	6738	348	NMA	363.3	40.423	5.763
6-5-73	150.1	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-6-73	160.6	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-7-73	149.7	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-8-73	154.4	2	6083	1160	7.99	ND	7263	464.2	NMA	384.3	40.874	5.836
6-11-73	124.4	2	10820	1220	7.62	9.40	11600	428	NMA	477	38.293	5.264
6-13-73	117.8	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 54 (continued). RUN NUMBER 8, ROTOR SPEED 275 RPM, TEMP. 10° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
6-14-73	137.6	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-18-73	138.5	8	14340	1810	7.42	5.4	15643	536.4	NMA	695	43.990	5.661
6-19-73	131.1	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-20-73	95.5	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-21-73	0	0	1333	1366	7.30	6.00	17740	533	NMA	370	42.502	5.184

Table 55. RUN NUMBER 9, ROTOR SPEED 380 RPM, TEMP. 10° C

Date	Waste added (g)	H_2O added (1)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
6-14-73	1713				ND	ND	ND	ND	ND	ND	ND	ND
6-18-73	126.6	8	9427	1107	7.17	8.50	8057	386.4	NMA	372.4	45.139	4.221
6-19-73	135	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-20-73	119.5	3	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-21-73	118.8	0	8640	920	7.60	8.9	8416	388.5	NMA	598	46.814	6.198
6-22-73	141.2	3	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-25-73	66.11	4	9580	993	7.47	9.40	11191	358	NMA	405	46.586	6.539
6-26-73	123.4	2.75	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-27-73	134.1	2	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-28-73	131.3	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-29-73	62.42	3	12290	11105	7.58	7.60	11684	416	NMA	423	44.936	5.433
7-2-73	0	6	11740	10500	7.53	9.20	12303	523	NMA	395	44.498	6.165
7-3-73	64.01	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 55 (continued). RUN NUMBER 9, ROTOR SPEED 380 RPM, TEMP. 10° C

Date	Waste added (g)	H_2O added (l)	Total solids (mg/1)	Total volatile solids (mg/1)	pH	Dis-solved oxygen (mg/1)	Chemical oxygen demand (mg/1)	PO_4 (mg/1)	NO_3 (mg/1)	Total nitro-gen (mg/1)	Car-bon (%)	Hydro-gen (%)
7-4-73	63.1	6	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-5-73	112.2	3	9390	8190	7.61	9.20	8819	458	NMA	329	44.802	5.981
7-6-73	110.2	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-9-73	112.7	4	6700	5180	7.85	8.5	7207	408.7	NMA	306.6	ND	ND
7-10-73	130.9	3	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-11-73	95.47	3	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-12-73	103.1	4	6790	5420	8.24	12.80	9134	435.6	NMA	400	40.993	4.957
7-13-73	113	4	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-16-73	113.6	3	11140	9670	7.73	5.9	ND	516	NMA	505	44.456	5.772
7-17-73	123.1	6	ND	ND	7.73	5.9	ND	ND	NMA	ND	ND	ND
7-19-73	0	3	10430	ND	7.60	9.0	13635	238.1	NMA	510	44.685	4.816

Table 56. RUN NUMBER 10, ROTOR SPEED 180 RPM, TEMP. 10° C

Date	Waste added (g)	H_2O added (l)	Total solids (mg/l)	Total volatile solids (mg/l)	pH	Dis-solved oxygen (mg/l)	Chemical oxygen demand (mg/l)	PO_4 (mg/l)	NO_3 (mg/l)	Total nitro-gen (mg/l)	Car-bon (%)	Hydro-gen (%)
6-21-73	1074.39		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-25-73	67.21	4	9580	993	7.19	6.70	9406	213	NMA	238	41.280	6.804
6-26-73	117.0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-27-73	131.7	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-28-73	141.6	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-29-73	57.79	3	7620	6830	7.31	6.30	10707	332	NMA	317	44.655	5.763
7-2-73	0	4	8960	8050	7.30	7.80	9117	390	NMA	383	44.838	5.601
7-3-73	73.56	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-4-73	0	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-5-73	128.4	3	9400	8420	7.26	6.80	7692	367.5	ND	329	43.451	5.281
7-6-73	118.5	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-9-73	128.5	4	5100	4090	7.69	5.5	6606	405.5	NMA	264.6	ND	ND
7-10-73	125.9	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 56 (continued). RUN NUMBER 10, ROTOR SPEED 180 RPM, TEMP. 10° C

Date	Waste added (g)	H_2O added (l)	Total solids (mg/l)	Total volatile solids (mg/l)	pH	Dis- solved oxygen (mg/l)	Chemical oxygen demand (mg/l)	PO_4 (mg/l)	NO_3 (mg/l)	Total nitro- gen (mg/l)	Car- bon (%)	Hydro- gen (%)
7-11-73	112.5	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-12-73	114.7	0	6990	5736	7.87	8.70	7813	449	NMA	442	ND	ND
7-13-73	116	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-16-73	126.6	3	9300	8147	7.45	9.9	9949	482	NMA	478	41.606	4.919
7-17-73	111.2	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-19-73	0	2	9920	ND	7.43	5.6	9817	ND	NMA	502	45.089	6.150
7-20-73	120.4	ND	9920	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-23-73	0	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-24-73	121.7	4	ND	ND	ND	7.4	ND	ND	ND	ND	ND	ND
7-26-73	115.6	6	10440	9090	7.40	11.1	12170	158.5	NMA	546	43.214	5.137
7-31-73	0	7	9600	8266	7.41	ND	10596	318	NMA	540	39.929	3.989
8-3-73	0	0	9880	8630	7.99	8.30	10910	248	NMA	533	43.531	5.679
8-6-73	0	4	9710	8350	7.88	5.70	10109	482	NMA	492	43.386	5.167
8-9-73	0	0	8747	7467	7.48	4.5	14639	463.2	NMA	260	42.287	5.107

SECTION VII

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SECTION VIII

APPENDIX---IRRIGATION STUDY

INTRODUCTION

The operation of spray-irrigation ditch material plays an important role in treatment of cattle waste in an oxidation ditch. This is necessary because cattle manure solids build up in the ditch until the ditch can only function as a holding tank. At this point, ideally sometime before, the ditch material must be removed, and spray-irrigation pumping is one such method. The mechanical ejection of ditch manure into the air by a sprinkler-irrigating pump provided the opportunity to examine the production of airborne bacteria. Aerosol monitoring indicated the relative degree of public health hazard associated with this method of handling waste from an oxidation ditch. For this study, samples of air were taken during irrigation in order to monitor the aerosolized microbes produced in such an operation.

RELATED STUDIES

Previous aerosol studies of operations similar to spray-irrigation have been done (22, 27, 28, 29, 30, 41). The similarity between these studies and ours was the production of a visible spray of sewage from trickling filters, aeration tanks, laboratory-generated sprays, and to other sprinklers. The differences, however, warranted carrying out our own study. For example, no other study dealt with cattle waste alone without the other additives of the sewage system such as plastics, paper, disinfectants, etc. The droplet size produced by the different operations varies due to pressure, spray nozzle size (19), and bubble size (22), which adds to the different conditions. In addition, we felt it was important to compare the bioaerosol production of the spray-irrigation to our central study of monitoring the bioaerosol from the oxidation ditch where the same system of sampling was employed; for example, the same samplers, location, procedure, and operator.

RESULTS AND DISCUSSION

The four studies during spray-irrigation are presented in Tables 57, 58, 59, 60. Each sampling time has been analyzed separately because daily and even hourly varying conditions prevented overall generalizations without first analyzing data under constant conditions. From Table 57 it can be seen that at the edge of the spray the airborne bacterial counts were lower than at distances farther downwind. The high counts at 82 meters downwind may have been due to an unnoticed wind change, an unknown spreading pattern which had a high concentration of bacteria at this distance and height, or an unknown factor. The counts of the sample taken after spraying were lower than all the counts except those taken at the edge of spray.

Data from Table 58, however, show no pattern of zonal concentration; but the total counts during spraying were higher, although not significantly (during - average of 3, before - 1, upwind - 0.4), than the counts before spraying and the counts upwind.

Table 58 shows that there was no difference in numbers of airborne bacteria between the 100- and 30-cm above-ground samples at any distance. There was no zonal concentration apparent, but comparing two 15 meter downwind samples it is found that the counts downwind increase when the wind increases. No indicator organisms (coliform or F. streptococci) were detected from AGI samples but were found on all exposed plates during irrigation--implying that particles from the ditch were airborne but not detectable in sizes that would penetrate the lung (1-5 microns). The total count, however, has increased from 0 before spraying to 30 CFU/1 during spraying, showing that some small particles are airborne. The after-spray counts show that the small and large fallout particles are quickly removed from or diluted out of the air.

The ditch liquid samples show the enteric indicator organisms to be approximately 300 times less than the concentration of the total number of organisms present. This same concentration proportion is borne out in the AGI samples of the air, but the open plates show proportionately higher concentrations of enteric organisms recovered. One explanation of this may be that since open plates collect particles that are falling, therefore large particles, each colony probably has many bacteria, thus making the actual number of bacteria present much more than counted. Also, since there are 300 times the number of total bacteria than enteric bacteria in the sprayed liquid, the chances of having many enteric organisms per falling particle are small. So the number of enteric colonies on the plates approximates the actual number. Unlike other air samples taken near the animal housing unit where F. streptococci outnumber coliform, the fallout plates show similar numbers of coliform and F. streptococci present. A speculated reason for this is that in spite of the fact that coliform organisms do not survive long when airborne (22), it is possible that they are protected by a coating of ditch material when they are in the fallout droplets, thus accounting for the coliform counts approaching those of the fecal streptococci.

The few samples on Table 59 show that when the large fallout particles are present in large numbers (overgrowth) the smaller, lung-penetrating sizes are not necessarily present (1 CFU/1).

Table 57. AEROSOLS FROM SPRAY IRRIGATION AT ROSEMOUNT - NOVEMBER 22, 1971

Time	Meters Downwind	Total CFU/1
During spray	0	0.4
During spray	61	15
During spray	82	120
During spray	113	25
After spray	82	4

Temperature:	-3° C
Wind:	8-16 km/hr
Sky.	overcast
Sampler:	AGI

Table 58. AEROSOLS FROM SPRAY IRRIGATION AT ROSEMOUNT - APRIL 18, 1972

Time	Meters Downwind	Total CFU/1
Before spray	0	1
During spray	37	3
During spray	52	4
During spray	67	2
During spray	98	5
	Meters Upwind	
During spray	37	0.4

Temperature:	8° C
Wind:	16 km/hr
Sky:	overcast 79% RH
Sampler:	AGI

Table 59. AEROSOLS FROM PIG WASTE SPRAY IRRIGATION - NOVEMBER 8, 1972

	Meters downwind	AGI: CFU/l			Plates-CFU/5 min. expos.		
		Total	Coli	F-Strep	PCA	L-EMB	m-ENT
Before spray	33	1	0	0	64	0	1
During spray	15	0	0	0	ovgr	ovgr	ovgr

Temperature:	10° C
Wind:	11 km/hr
Sky:	overcast
Sampler:	AGI, plus exposed plates at ground level

The data collected by others add additional light on the subject and support to the data collected in our experiment.

Albrecht (29) found when sampling with both a Wells centrifuge and midget impinger downwind from a trickling filter sewage treatment plant that very few coliform organisms were recovered, but growth on nutrient agar showed recovery from 0.07 to 5 CFU/l at the edge of the trickling filter, decreasing to 0.2 to 2.7 CFU/l at a distance of 15 meters. He noticed an increase in counts downwind with increasing wind velocity.

Randall (27) found in sampling air downwind from activated sludge units with an Anderson sampler that on the average 17 CFU/l of particle size less than 5 microns were found downwind of the operation (6% of which were Klebsiella). He noted that as the wind increased so did counts and also there was a rough correlation between higher counts at higher R.H. He concluded by saying there existed a definite possibility of airborne infection from activated sludge units.

Adams (30) sampled the air with EMB in an Anderson sampler near a trickling filter. Without classifying bacteria any further than

Table 60. AEROSOLS FROM SPRAY IRRIGATION AT ROSEMOUNT MARCH 26, 1973

	Down wind (m)	AGI: CFU/1						Agar plates (ground) CFU/5 min. exposure		
		30 cm			100 cm					
		Total	Coli	F-Strep	Total	Coli	F-Strep	PCA	L-EMB	m-ENT
Before spray	0	1	0	0	4	0	0	ovgr*	0	0
During spray	15	2	0	0	6	0	0	348	28	27
During spray	15**	23	0	0	30	0	0	>2000	98	188
During spray	33	23	0	0	26	0	0	221	1	5
After spray	50	0	0	0	0	0	0	ovgr*	0	0
	Up-wind									
During spray	33	2	0	0	0	0	0	77	0	1

Bacterial counts of ditch liquid collected during irrigation:

92×10^6 total aerobic bacteria per ml ditch
20×10^4 Coliform bacteria per ml ditch
32×10^4 F. Streptococci per ml ditch

* ovgr denotes overgrowth with a spreading organism.

** during this sampling the wind speed was greater than the other 15 meter downwind sample.

Temperature:	11° C
Wind:	8 km/hr
Sky:	Clear, 57% RH
Sampler:	Samples with AGI were taken simultaneously at two heights, 30 and 100 cm, plus exposed plates on ground.

counting colonies on media, he found 19 CFU/l at 15 meters downwind, 0.9 CFU/l at 42 meters downwind, and 0.003 CFU/l at 1280 meters downwind. He observed greater recoveries with high wind velocity, high R.H., and darkness.

Napolitano (28) studied concentrations of aerosols generated by activated sludge plants using EMB in an Anderson sampler. The report says that sampling counts were highest at the aeration source where a noticeable spray was produced. Here 8 CFU/l were obtained on EMB (no further selection was done; however, it was stated that *Aerobacter aerogenes* was predominate). At 15 and 30 meters downwind 0.18 CFU/l was found.

Merz (41) found when sampling with an impinger downwind from a sprinkler discharging settled sewage onto a golf course that he detected coliforms in the air only at distances where spray could be felt. He concluded that there was no hazard except that of direct contact with unevaporated droplets.

Higgins (22) found in a laboratory study of generated bioaerosols by aeration and the production of bubbles that the production of viable bacterial aerosols by bursting bubbles is highly dependent upon species. *E. coli* had a *very* low aerosolization rate. In addition, he found that the aerosol production depended on composition of aeration liquid, wind velocity, and concentration of cells. He could not make any statement on the effects of air and water temperature or R.H.

Ladd (26) found when sampling near a sewage pre-aeration treatment with the Anderson-sieve sampler he was able to pick up a tracer organism, *Bacillus subtilis* var. *gobgii*, that was poured down the laboratory drain (approximately 8 km from the sewage plant). He was able to detect the organism at a maximum concentration of 0.008 CFU/l (15 organisms per 60 ft^3) 5 hours after the pouring but detected none after the eleventh hour. A concentrated suspension solution of 4.5 liters was poured into the drain - approximately 10^6 (our estimate) organisms per ml. He also found 8 times as many bacteria were emitted by the pre-aeration tank as measured by upwind controls. He concluded that harmful bacteria were being emitted from the treatment plants and could be harmful to operators and others in the area.

CONCLUSION

The assessment of the public health hazard of spray-irrigating liquid from an oxidation ditch involved many factors, many of which are unknown. But two likely ways exist in looking at the problem. One method is by examining the greatest bioaerosol load produced by any such operation, fitting into the equation the known infectious doses of bacteria potentially inhabiting the aerosol and survival curves of airborne bacteria, also including the expected contaminated air a person breathes (15,000 liters per day) (44), then extrapolating to find the probability of infection. A second method would be to compare disease rates produced from an operation of similar bioaerosol loads; for example, animal housing units or, in the case of sewage, comparison with a hospital.

In dealing with cattle waste an evaluation by the first method is difficult because most studies on, for example, airborne survival have been done on human airborne disease,not cattle. What is known in this area, however, is that a dose as low as one tubercle bacillus can cause tuberculosis, whereas 10^4 pneumococci are needed to infect a mouse by airborne route (44). In addition, very little is known of the respiratory infectious dose of enteric bacteria which are the potential hazards being looked at in irrigating an oxidation ditch. This lack of information with which to analyze the hazard leads to the alternate analysis of comparing similar situations.

In none of the bioaerosol studies with the AGI during irrigation were any enteric organisms present (less than 1 CFU/l), and total counts increased from 1 CFU/l before spraying to a maximum of 120 CFU/l and an average of 20 CFU/l during and downwind of spray. Other cited researchers present this same number when sampling downwind in the vicinity of sewage spray. The counts of samples taken seconds after spraying stopped are the same as before spraying samples, indicating that there is no buildup of bacteria. The previously reported sampling of animal housing units frequently have enteric F. Strep.; also, the average Rosemount animal housing unit total count is 161. In addition, the samples of a dairy barn show averages of 196 total aerobic bacteria and 0.5 F. Strep. Therefore, the relative hazard of spraying compared to confinement housing is small as far as cattle health and people working in the housing areas are concerned. As far as the respiratory hazard of workers in the area there is no denial of the increase in bioaerosol from the ditch during spraying in the immediate area, but this hazard is present for a short while. Therefore, it is recommended that, during spraying, workers in the area wear protective masks. A more possible danger is through contact with large droplets of spray which are visible during the operation. Diseases can arise in the nasal mucosa, tonsils, or respiratory mucosa of the upper respiratory tract through inhalation of large particles (37). Again, a mask in the areas during spraying is recommended.